MICROBIOLOGICAL REVIEWS, Mar. 1981, p. 123-179
0146-0749/81/010123-57$02.00/0

Vol. 45, No. 1

# The Caulobacters: Ubiquitous Unusual Bacteria

JEANNE STOVE POINDEXTER

*The Public Health Research Institute of the City of New York, Inc., New York, New York 10016*

## INTRODUCTION

Distinctive in morphology and possibly as ubiquitous in the biosphere as water itself, the caulobacters have excited greater interest by their developmental peculiarities than by their presence in practically every type of aquatic community and in many soils. Their numbers and possibly also their biogeochemical contributions are often overshadowed by those of "morphologically nondescript" (266) pseudomonads. Nevertheless, this easily recognized bacterial group offers considerable potential for providing new insights into microbial activities in environments where changes are not dramatic and rapid, but which, due to their vastness on the surface of the earth, are undoubtedly significant in the management of life's substrates.

The characteristic caulobacter cell (Fig. 1) is gram negative and possesses at least one appendage derived from the cell envelope; the appendage is regarded as a stalk (86) when it carries adhesive material at the distal end and as a pseudostalk (213) when it is not adhesive. These appendages represent one type of "prostheca," a term proposed by Staley (258) to designate any bacterial appendage or protrusion derived at least in part from the cell envelope. In two genera, *Caulobacter* and *Asticcacaulis* (the subjects of this review), the prostheca appears to consist exclusively of envelope components; through most or all of its length, cytoplasmic elements, such as ribosomes or nucleoplasm, are not detectable by microscopy or by chemical analysis. Other slender, cylindrical prosthecae, e.g., in *Ancalomicrobium*, *Prosthecobacter*, *Hyphomicrobium*, and *Rhodomicrobium*, possess cytoplasmic elements. A further structural peculiarity of the caulobacter prostheca is the bands which are present at irregular intervals along the appendage. It was earlier proposed that each band indicated a cell division event in the stalked cell (262), but, as presented later in this review (see Fig. 5), this is not the case, since several bands can appear in the stalks of cells before the first fission.

Equally distinctive of caulobacters is the morphology of the dividing cell (Fig. 2). In both genera, the typical division figure is asymmetric because at or near one pole of the cell there is at least one prostheca and at or near the other pole is a single flagellum. Fission consequently results in morphologically and behaviorally different siblings–one prosthecate and nonmotile and the other flagellated and motile, the swarmer cell. Both progeny, however, are capable of attachment to solid substrates by means of adhesive material present at the stalk tip (in stalked *Caulobacter* cells) or at the cell pole (in *Caulobacter* swarmers and in *Asticcacaulis* cells).

In discussion of surface morphogenesis in such species as *Caulobacter crescentus*, mention is occasionally made of a "reversal of polarity." This view seems misleading, however, when it is recognized that the same morphogenetic sequence occurs at every pole, and polar morphogenesis can be viewed as a process symmetric across a cell region that begins as a division site. Followed through three generations, the morphogenetic events are as follows. At the conclusion of generation 1, fission occurs at this site, yielding two new cell poles. During the D period (84) of generation 2, each of the new poles becomes flagellated and extrudes attachment materials (holdfast material and sometimes also pili). By the onset of the C period of generation 3, the flagellum has become inactive and may be shed, and stalk development is initiated. Thus,

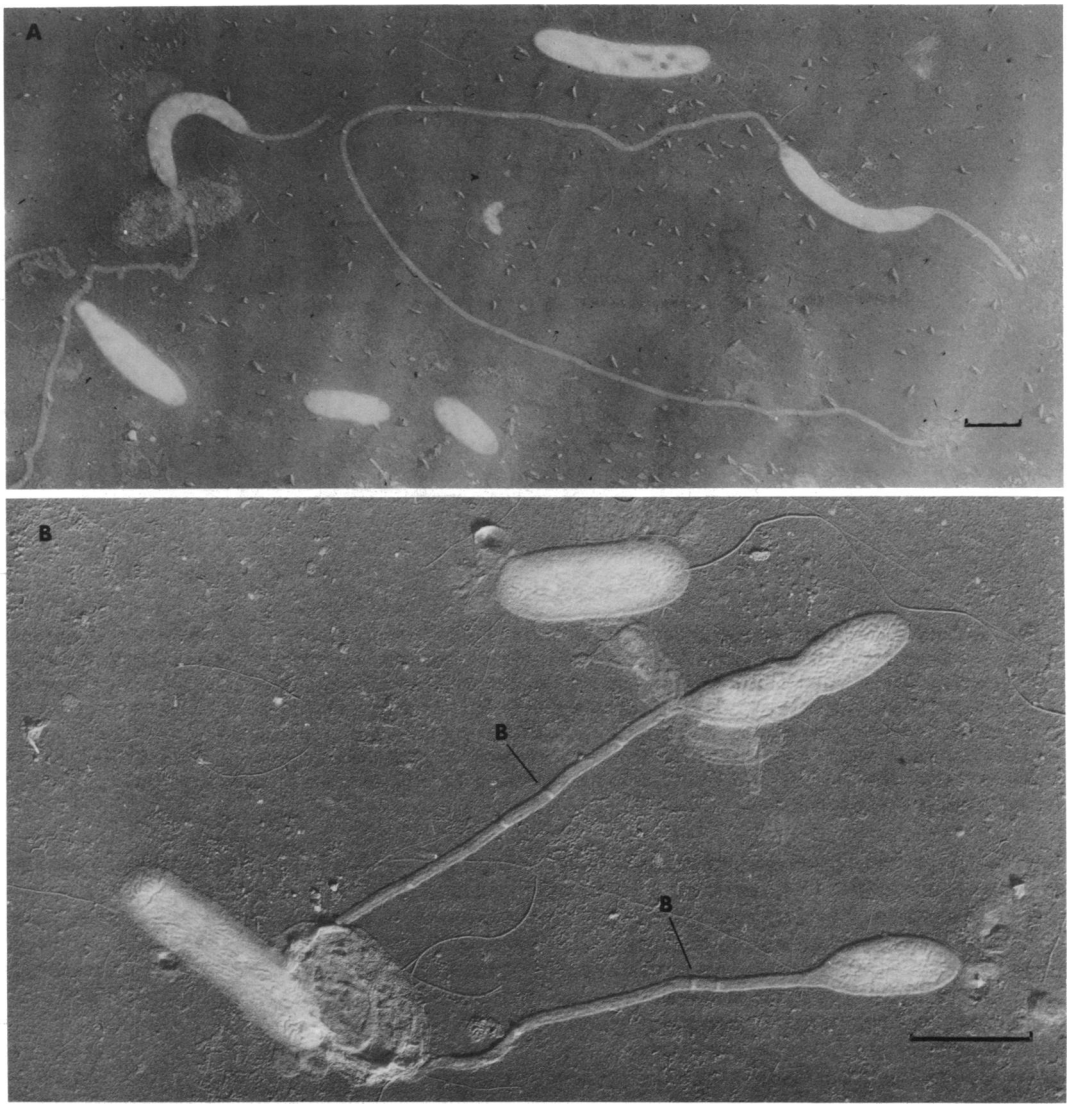


FIG. 1. *Caulobacter cells in enrichment cultures. (A) Vibrioid stalked cells exhibiting one long stalk and one shorter stalk on each cell. (B) Bacteroid stalked cells associated with other bacterial cells, one of which has lysed. The bands (B) visible at intervals along each stalk are also present in (A); absent from other prosthecate bacteria, the bands are diagnostic of caulobacters in wild populations. Each marker is 1 μm.*

both the sequence and the period of development within each cell cycle are the same for both poles formed in generation 1. The difference in the time courses of their development occurs in generation 2, in which the onset of the C period occurs earlier in the stalked sibling than in the swarmer; the difference in their cycle times for generation 2 is equal in duration to the period of active swarming in the flagellated sibling.

During the past 15 or so years, most of the experimental studies with caulobacters have been directed toward elucidation of the structural and biochemical events of the swarmer cell cycle (Fig. 2). The basic morphogenetic sequence—loss of motility, initiation of stalk development, development of the division site, and activation of the new flagellum—were inferred by Henrici and Johnson (86), Houwink and van Iterson (96), and Masuda (175) and later demonstrated by Stove and Stainer (269) through observations of microcultures and of synchronously developing swarmer populations. More recent studies have further demonstrated that

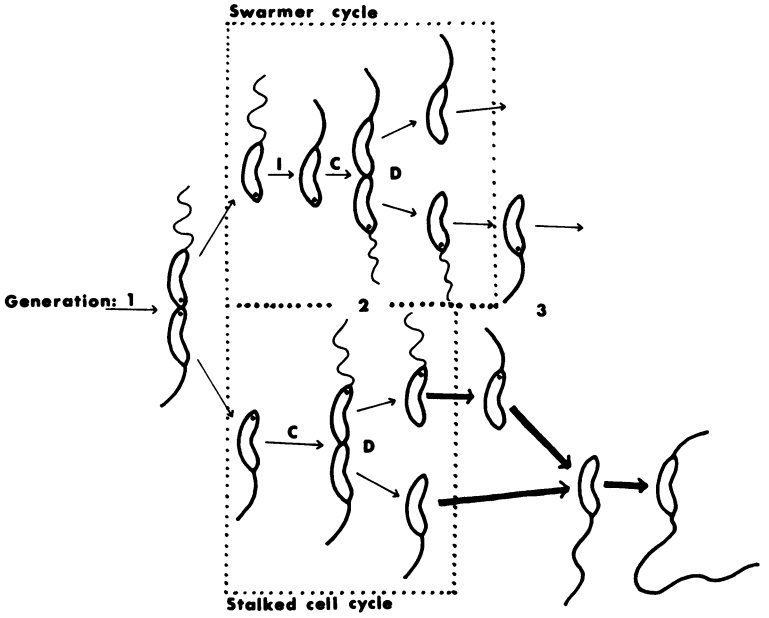


FIG. 2. *Development and reproduction in Caulobacter. Cell division results in the production of morphologically different siblings; one is stalked and nonmotile, whereas the swarmer possesses a single flagellum and is motile. The swarmer sheds its flagellum and, at the previously flagellated site, develops an outgrowth of the cell envelope, the prostheca or stalk. Diamonds (◆) follow the symmetry of polar development across a division site through generations 1, 2, and 3. I, C, and D indicate the procaryotic cell cycle periods (84). Heavier arrows indicate morphogenetic events under conditions of nutrient exhaustion; each type of cell develops a stalk that may become several times longer than the cell, often accompanied by development of a second stalk at the opposite pole.*

certain biosynthetic processes, most notably deoxyribonucleic acid (DNA) synthesis, fluctuate during the swarmer cycle; these reports are reviewed in Experimental Studies of Cellular Differentiation, below. The isolation of morphogenetic mutants and the development of transductional and conjugational systems for genetic studies with caulobacters are promising developments for the analysis of regulation of this sequence.

In natural populations and in artificial media with growth rate-limiting concentrations of required nutrients (bold arrows in Fig. 2), caulobacter prosthecae extend to many times the cell length; at least in *Caulobacter*, the development of a stalk at the second pole is common in such populations. This often-repeated observation implies that prosthecal development may promote survival of caulobacters in dilute environments. However, investigation of the possible contribution of the prostheca to physiological activities of the cell has so far been limited to studies of transport of nutrients by prosthecae artificially detached from the cells. I have nevertheless attempted to correlate information from various types of experimental studies with observations of caulobacters in nature. In the final section (Ecological Implications) I offer an interpretation of the possible significance to the existence of caulobacters of traits that these bacteria exhibit in laboratory cultures and propose a role for the caulobacters as mineralizers in environments that support only sparse microbial populations.

This review is intended to cover the literature from ca. 1964 through early 1980. I should be grateful for references to any significant contributions that have been overlooked.

## NATURAL DISTRIBUTION

As noted by Henrici and Johnson (86), caulobacters can be observed on slides immersed in any kind of freshwater, "even in running tap water," at any time of year. The same is proving true for neutral soils (13, 90, 130, 190, 191, 293). Even many who are not seeking caulobacters find stalked bacteria (9, 25, 44, 50, 64, 78, 96, 106, 108, 126, 151, 158, 159, 181, 187, 196, 216, 275, 288). Prior to Henrici and Johnson's systematic examination of the flora of submerged slides, caulobacters had been isolated on at least three occasions: by Loeffler in 1890 (165), Jones in 1905 (121), and Omeliansky in 1914 (202), in each case from water samples. The appendage

was interpreted as a peculiar flagellum, an interpretation that has been repeated by later workers as well (288, see also reference 94; 106, see also reference 107).

The caulobacter appendage is not visible on living cells by ordinary light microscopy, and unmordanted staining procedures with dried cells are not dependable means of making the stalks visible. The introduction of electron microscopy to the study of bacterial cells revealed the details of the structure of the caulobacter stalk and its adhesive material, as well as its relationship to the flagellum (96). After the studies of Houwink and van Iterson (96), establishment of the identities of all types of prosthecate bacteria has involved electron microscopic examination of isolates.

At present, examination of natural materials for the presence of caulobacters depends primarily on phase-contrast microscopy, by means of which the stalks of living cells are readily visible to the experienced observer. Probably as a consequence of the widened use of this type of microscopy, the frequency of sightings of caulobacter in surveys of natural materials has risen in the past 2 decades, justifying the opinion of Henrici and Johnson that they are, indeed, ubiquitous; in some samples, they are the major types of aerobic chemoheterotrophic bacteria.

## Detection and Enumeration

**Freshwater and soil.** Over a period of 2 years (1965 to 1967 [130]), Belyaev and Krasil'nikov surveyed natural and managed (reservoir) bodies of freshwater and a wide variety of soils for the presence and numbers of caulobacters (11–13, 130). During the course of their studies, they accumulated 127 isolates that they used in physiological studies (14, 15, 132, 133) with the aim of clarifying the relationship between the distribution of caulobacters and physical and biotic factors.

Probably the most significant aspect of their studies, with respect to both detection and enumeration of caulobacters in soil and water, was their demonstration that viable caulobacters can be detected in many samples only if the sample is diluted. By using extinction-dilution procedures for estimating numbers of viable bacteria, they discovered that caulobacters were often undetectable in lower dilutions (1:10, 1:100) of the sample, but readily detectable in higher dilutions (1:1,000, 1:10,000) (11). In soil samples, Belyaev (12, 13) found that the numbers of caulobacters decreased significantly if the soil sample was allowed to dry; the discernible numbers were increased significantly by grinding the soil gently and then shaking it in water before dilution into enumeration broth. Dilute peptone (0.01%) or diluted meat-peptone (1:100) broth proved the most suitable medium for the enumeration of caulobacters.

Krasil'nikov and Belyaev found caulobacters in practically every type of sample, with the exception of water samples from Antarctica (13). In general, the presence, number, and proportion of caulobacters among chemoheterotrophic bacteria in water samples were inversely related to the total heterotroph count and to the density of suspended particulate matter (11, 13). (It is unfortunate that none of the studies of the distribution of caulobacters in natural waters has included determination of the ambient concentration of organic carbon; organic content has been inferred on the basis of total heterotroph counts, phosphate concentration, or transparency.) In waters of highest transparency (4 of 11 stations sampled), caulobacters predominated over other heterotrophs by 7- to 100-fold; in those samples, the noncaulobacter counts were less than 200/ml. Caulobacter counts per milliliter of surface film were generally about 10 times higher than caulobacter counts in subsurface samples, and caulobacters were often absent from sediment samples (11). A more recent study, using partially submerged stationary slides, claimed that caulobacters were particularly numerous in the upper 15 $\mu$m of reservoir water (228).

Three environmental factors were identified in Krasil'nikov and Belyaev's survey as especially favorable for development of caulobacters among the soil microflora: neutral or slightly acid pH, high moisture content, and low organic content (15, 130). In such soils, as many as 600,000 viable caulobacters were found per g of soil (15). In contrast to observations on water samples, there was not an inverse correlation between total heterotroph counts and caulobacter counts in soil samples (13).

In a seasonal study by Staley of a polluted stream (259), prosthecate bacteria (predominantly caulobacters except in the December sampling) accounted for, at most, 1% of the total viable heterotrophs. The lowest caulobacter counts were observed in September, at a time when the samples were visibly turbid and total heterotroph counts were maximal (some greater than $10^7$/ml). The highest numbers and proportions of caulobacters were observed in the spring, when total heterotroph counts were less than $10^6$/ml. In a survey of five Australian freshwater bodies of different trophic states (defined by phosphorus content), Staley et al. (264) found prosthecate bacteria (again, predominantly caulobacters) present in higher numbers and proportion (ca. 36%) in the mesotrophic lake than

in the oligotrophic lakes or eutrophic ponds. It was inferred that nutrients limited caulobacter development in oligotrophic lakes, yet in mesotrophic habitats the caulobacters still had a competitive advantage over heterotrophs that predominated in eutrophic habitats.

Prosthecate bacteria, including caulobacters, appeared as a significant proportion (generally about 2%, but as high as 10%) of the bacterial populations of pulp mill waste lagoons (266). The waters of the lagoons are routinely adjusted to neutrality and vigorously aerated, both of which treatments undoubtedly improve their suitability for the growth of prosthecate bacteria. *Caulobacter* was present in the majority of samples, in proportions that implied numbers as high as $10^6$/ml. Proportions were determined by direct microscopic counts, a technique that Staley has noted (259) underestimates the number of prosthecates, such as *Caulobacter* and *Hyphomicrobium*, which bear appendages only at certain developmental stages. It is also probable that direct differential microscopic counts especially underestimate caulobacters, since (i) the proportion of appendaged cells is lowest when the population is most actively reproducing (77, 97, 98, 122, 152, 222, 227, 237, 247) and (ii) higher nutrient concentrations that stimulate the growth rate also typically reduce the lengths and incidences of the appendages (98, 152), making them more difficult to visualize. The incubation of diluted samples in dilute media thus seems preferable as a survey procedure to avoid underestimation of prosthecate populations. A precise enumeration procedure has yet to be devised.

**Seawater.** Caulobacters appear to be relatively abundant in the open ocean, and less so in nearshore waters. No halophilic caulobacters have been reported. Jannasch and Jones (106, 107) found that caulobacters could account for 13 to 45% of the bacterial population of some ocean samples. As in freshwater, the proportion of caulobacters was inversely related to total heterotroph counts. Leifson et al. (161) found stalked bacteria only rarely in their samples of water from Long Island Sound, Narragansett Bay, and the Atlantic Ocean, but succeeded in obtaining at least 4 among their 600 isolates. Mishustina et al. (179) encountered *Caulobacter* spp. frequently in ocean samples, from the Barents Sea to subtropical waters of the Atlantic and from superficial layers down to depths of 5,500 m; they concluded that prosthecate bacteria constituted a significant part of the microbial population of the sea. Austin et al. (9) found nonmotile caulobacters to be one of three generic groups accounting for nearly all of the chemoheterotrophic bacteria of Tokyo Bay; the other principal types were the *Acinetobacter-Moraxella* group and *Pseudomonas* spp.

**Attachment and community associations.** Caulobacters have been detected in waters and soils by several workers whose studies did not include enumeration. A typically successful means of detecting prosthecate bacteria in a body of water or in soil is to submerge or bury glass slides or cover slips (20, 86, 175, 228), Perfil'ev capillary tubes (130, 216), or plastic-coated electron microscope grids (92, 179). Caulobacters, in particular, are suitably "baited" in this fashion and attach to the substrate. There are three types of evidence that it is usually the swarmer cell that attaches. (i) The swarmer is motile and more likely to collide with the substrate; it is also possible for the swarmer to be chemotactically attracted to the accumulating film of nutrients (174) on the substrate. Motility also seems important in providing the mechanical force necessary to overcome electrical repulsion between substrate and cell (38). (ii) Stalked cells are not seen in the early stages of community development on the substrate (37); it has not yet been determined whether this is due to their appearance as a seral stage of the microbial succession that occurs on substrates or whether their morphogenesis in such environs occurs at so low a rate that their stalks are not apparent until a day or longer after attachment of the cells. (iii) Newton (193) has demonstrated that the number of cells that attach to a cover slip in 1 min correlates well with motility estimations based on observation of wet mounts. This implies that brief submersion or burial, followed by fixation or immediate microscopic counting, will underestimate or fail to detect caulobacters. For detection, it would seem advisable to remove the substrate to a sterile medium and allow incubation time sufficient for stalk development by attached swarmers. Such a procedure would conceivably permit detection of caulobacters even among the first bacteria that attached to the substrate.

Inanimate surfaces, including soil particles (13, 293), are only one type of substrate for attachment of caulobacters. Other microorganisms also serve (64, 130), particularly algae (11, 13, 24, 80, 190, 192, 289–291). In several instances, it has been noted that caulobacters seem to attach particularly to dead or moribund cells (93, 133, 175, 218; see Fig. 1). This could indicate a causal role of the caulobacters in the deterioration of the substrate organism (93, 95, 97, 175, 289), a lack of inhibition of their attachment by the substrate organism due to its failing metabolic activities, or simply an accumulation of

caulobacters on nongrowing cell surfaces. In either of the latter two cases, the attaching caulobacters can be viewed as having located themselves at the source of a supply of nutrients leaking from the dying substrate. It is difficult to demonstrate enhancement of viability of caulobacters in two-membered cultures, since a substrate cell heavily invested with caulobacters will plate as a single colony-forming unit (see, e.g., Fig. 21, 22, and 24 of reference 218). Nevertheless, Krasil'nikov and Belyaev (133) were able to demonstrate that the presence of *Azotobacter chroococcum* in a medium lacking combined nitrogen was a suitable substitute for $NH_4Cl$ or yeast extract in supporting maximum growth of two species of *Caulobacter* (*C. crescentus* and *C. vibrioides*). The association seemed mutually beneficial, since the azotobacters grew more rapidly in the presence of the investing caulobacters than in single-membered culture.

Association with caulobacters has also been found beneficial to algae. Bunt (24) found that a *Caulobacter* contaminant of cultures of a soil *Nostoc* ensured development of single hormogonia into healthy, heavily pigmented trichome masses. The beneficial effect was attributed to production by the *Caulobacter* cells of substance(s) with auxin activity and to stimulation of phycoerythrin production in the cyanobacteria. Frequent detection of caulobacters in algal cultures (24, 80, 192, 289) suggests that the possibility of mutual benefit should also be explored with respect to associations of algae and caulobacters.

### Enrichment and Isolation

The procedure devised by Houwink (93) for enrichment and isolation of caulobacters from water was to add peptone to a concentration of 0.01% and to incubate without agitation; caulobacters accumulated in the surface film and were isolated by streaking a sample of the film onto dilute peptone agar. The method has proved generally suitable for the enrichment and isolation of chemoheterotrophic prosthecate bacteria (15, 88, 91, 215, 218, 258, 261). To avoid overgrowth by bacteria that form large colonies or that spread over the agar surface, it was found advantageous to remove the small, circular, convex, noniridescent, somewhat hyaline- or crystalline-appearing colonies to a further plate as patch inocula (218, 268); growth in the patches could then be screened microscopically for stalked bacteria, and ample incubation time could be allowed for development of readily discernible stalks.

Enrichment of marine caulobacters has usually proceeded by essentially the same means as used for freshwater isolates (161, 218). In one case, however, caulobacters were enriched and isolated without specific intention to obtain prosthecate bacteria. Murakami et al. (187) prepared seawater enrichment cultures for paraffin-degrading bacteria. Two types were eventually isolated: *Caulobacter* and *Flavobacterium*. Subsequent studies demonstrated that both types readily utilized paraffins ($C_{12}$ through $C_{16}$) provided as the sole source of carbon. This seems to be the only report as yet of hydrocarbon-utilizing caulobacters, but it implies that the degradative capabilities of this group have not yet been fully appreciated, particularly in marine environments.

In summary, it has not yet proved possible to promote the development of caulobacters over nonprosthecate chemoheterotrophic bacteria by means other than using generally utilizable nutrients at low concentrations. The procedure still depends on allowing their accumulation in the surface film and on microscopic observation of the presence of stalked cells in an isolated clone. The procedures that have been successful are those that selected for aerobic oligoheterotrophs.

## TAXONOMY

### Relationship of *Caulobacter* and *Asticcacaulis* to Other Bacterial Groups

When compared with all bacteria, the caulobacters seem most similar—in morphology, habit, and natural distribution—to other prosthecate bacteria (182, 235, 260, 267). However, it is not yet clear whether the outward similarities among prosthecate bacteria are a consequence of common descent or of analogous morphological adaptations to similar niches. Although Staley's definition of a prostheca (258) as an appendage derived at least in part from the cell wall requires some homology of structure and development of this organelle, the evolutionary origins of the appendages of the several prosthecate groups may have been as independent as those of the *Caulobacter* and *Gallionella* stalks (134, 225).

*Prosthecobacter* (261) in particular appears similar to *Caulobacter* and *Asticcacaulis* and has been referred to informally as a caulobacter (45, 261). In this genus, proposed since the eighth edition of *Bergey's Manual of Determinative Microbiology* (23), fission is symmetrical and results in two nonmotile, prosthecate siblings; it is probably identical with one of the stalked bacteria described, but not isolated, by Henrici and Johnson (86). The nonbudding, unbanded,

cylindrical prostheca of *Prosthecobacter* is of greater diameter than the banded appendage of *Caulobacter* and *Asticcacaulis* and contains ribosomes, rather than membranes, throughout its length (261). It is, accordingly, an extension of the protoplast rather than exclusively an envelope-derived organelle. Structurally and developmentally it is a different kind of appendage. More importantly, the absence of flagella in *Prosthecobacter* and the symmetry of cell division imply not only a developmental sequence different from that of motile caulobacters, but also a distinctly different relationship to the environment. Thus, the similarity of this organism to motile caulobacters seems largely superficial. Nevertheless, symmetrical cell division is not impossible in *Caulobacter*, and *Caulobacter* cells with secondary stalks (78, 80, 86, 131, 175; J. L. Stove, Ph.D. thesis, University of California, Berkeley, 1963; see also Fig. 1A) would not be distinguishable from cells of *Prosthecobacter* (or those of "*Thiodendron latens*" [p. 145 of reference 23]) in natural materials.

The DNA base composition of *Caulobacter* spp. was determined to be 62 to 67 mol% guanine plus cytosine, and that of *Asticcacaulis excentricus* was determined to be 55 mol% guanine plus cytosine (218). The DNA of most of the other prosthecate bacteria is in the range 60 to 70 mol% guanine plus cytosine (168, 169, 184, 215, 258, 263); only *Prosthecobacter* DNA contains as little as 56 mol% guanine plus cytosine (45). Although this range is as narrow as those in other monoflagellate gram-negative genera, such as *Pseudomonas* (167, 173, 210), it is not correlated with significant DNA homologies among prosthecate bacteria (184, 185). However, a more sensitive test for genetic similarity, viz., homology between ribosomal ribonucleic acid (RNA) and DNA, did reveal a closer relationship between *Hyphomicrobium* and other prosthecate bacteria (*Hyphomonas*, *Rhodomicrobium*, *Prosthecomicrobium*, and *Caulobacter*) as well as nonprosthecate photosynthetic bacteria (*Chromatium* and *Rhodopseudomonas*) than between *Hyphomicrobium* and 14 nonprosthecate, non-photosynthetic genera, including *Pseudomonas* (182).

In a study of homology among insertion sequence elements IS1, IS2, and IS5, Nisen et al. (198) found that of eight bacterial genera tested (*Escherichia*, *Salmonella*, *Citrobacter*, *Serratia*, *Erwinia*, *Pseudomonas*, *Klebsiella*, and *Caulobacter*), only *Caulobacter* (*C. crescentus*) lacked homology with the insertion sequence elements. Similarly, *Caulobacter* (*C. crescentus*) was found by Nakamura et al. (188) to lack DNA homology with messenger RNA for a major outer membrane lipoprotein of *Escherichia coli*. This lack was also exhibited by the genera *Pseudomonas*, *Acinetobacter*, *Myxococcus*, and *Proteus*. Of the five genera, only *Proteus* exhibited (relatively weak) serological cross-reaction between its envelope and anti-*E. coli* lipoprotein serum. Both of these comparative molecular studies imply a significant evolutionary distance between *Caulobacter* (*C. crescentus*) and the *Enterobacteriaceae*.

Evidence of the relationship of caulobacters to the pseudomonads, based largely on the presence in both groups of the Entner-Doudoroff pathway for intermediary metabolism of carbohydrates (218), has not been further explored with respect to the features found suitable for subgeneric classification of *Pseudomonas* (265).

### *Caulobacter* and *Asticcacaulis* Species

Caulobacters in which cell division is typically asymmetric are currently classified in two genera: *Caulobacter* Henrici and Johnson 1935 and *Asticcacaulis* Poindexter 1964. The principal distinction between these two genera is the location of adhesive material on the outer tip of the prostheca in *Caulobacter*, but on the cell pole and not the prostheca in *Asticcacaulis*.

Eleven species (Table 1) have been organized as taxa of *Caulobacter* (86, 218), of which only 10 were included in *Bergey's Manual of Determinative Microbiology* (219). *Caulobacter variabilis* was not included because only one isolate had been obtained at the time the *Caulobacter* section of the manual was prepared. However, two isolates were later obtained from soil (134), and the taxon appears to be justified.

Krasil'nikov and Belyaev (134) were able to assign 99 of their 127 isolates to seven of the previously described species groups, in some cases proposing new subspecies. They proposed eight new species to accommodate their remaining 28 isolates. Gromov (80) studied nine isolates from algal cultures, including Zavarzina's (291), which proved so different from each other that all were regarded as varieties of the single species then available, *C. vibrioides*.

Marine isolates obtained by Jannasch and Jones (106, 107) were not described with respect to their morphology, pigmentation, or nutritional requirements. Thirteen isolates obtained by Poindexter (218) from filtered, stored seawater were classified as *Caulobacter maris* and *Caulobacter halobacteroides*, the only two species of marine caulobacters so far designated. With respect to nitrate reduction and sugar utilization, the isolates of Leifson et al. (161) appeared intermediate between these two species. The identity of the isolates of Austin et al. (9) as

TABLE 1. *Species established or proposed for Caulobacter and Asticcacaulis*

| Species | No. of described isolates | Morphology[a] | Non-morphological traits | | |
|---|---|---|---|---|---|
| | | | Pigmentation | Requirement(s) | Other distinction |
| *C. vibrioides* (87) | 30 | Vibrioid | None, yellow | Vitamin $B_2$, often also others | |
| *C. henricii* (218) | 10 | Vibrioid | Yellow, orange | Vitamin $B_{12}$ | |
| *C. intermedius* (218) | 1 | Vibrioid | None | Biotin and others | |
| *C. crescentus* (218) | 7 | Vibrioid | None | None | |
| *C. robiginosus* (134) | 1 | Vibrioid | Red-brown | Amino acids | Sucrose +,[b] lactose − |
| *C. rutilis* (134) | 2 | Vibrioid | Red-brown | Amino acids | Sucrose −, lactose + |
| *C. subvibrioides* (218) | 21 | Subvibrioid | None, dark orange | Unidentified (not B vitamins) | |
| *C. fusiformis* (218) | 3 | Fusiform | Dark yellow, dark orange | Unidentified | |
| *C. rossii* (134) | 1 | Fusiform | None | Unidentified | |
| *C. kusnezovii* (134) | 9 | Fusiform | Red-brown | Unidentified | |
| *C. leidyi* (218) | 1 | Fusiform | None | None | |
| *C. bacteroides* (218) | 66 | Bacteroid | None,[c] yellow, orange | None[c] or unidentified | |
| *C. metschnikovii* (134) | 2 | Bacteroid | Yellow-orange | Not reported | |
| *C. fulvus* (134) | 2 | Bacteroid | Yellow-brown, red-brown | Unidentified | |
| *C. flexibilis* (134) | 2 | Bacteroid, curved | None | Unidentified | |
| *C. glutinosus* (134) | 9 | Not described | None | Not reported | Colonies adherent |
| *C. halobacteroides* (218) | 1 | Bacteroid | None | Unidentified | Marine, amino acids + |
| *C. maris* (218) | 1 | Bacteroid | None | Unidentified | Marine, amino acids − |
| *C. variabilis* (218) | 3 | Bacteroid, stalk polar or subpolar | None, red-orange | Unidentified | |
| *A. excentricus* (218) | 5 | Bacteroid, subpolar prostheca | None | Biotin | |
| *A. biprosthecum* (152, 215) | 1 | Bacteroid, one or two lateral prosthecae | None | Biotin | |

[a] Vibrioid, Cell tapered, long axis distinctly curved; subvibrioid, cell tapered, long axis not strongly curved; fusiform, cell tapered, long axis not curved; bacteroid, poles rounded, long axis not curved.

[b] +, Utilized as carbon source; −, not utilized.

[c] Krasil'nikov and Belyaev assigned six isolates able to grow on glucose minimal medium without growth factors to this group, as *C. bacteroides* subsp. *modicus*. All six strains of one collection (218) were brightly pigmented, whereas all 60 strains of the later collection (134) were colorless.

*Caulobacter* is uncertain, since they were described as not motile.

All 19 *Caulobacter* species are listed in Table 1, along with the 2 species of *Asticcacaulis*. As indicated by the columns in Table 1, the primary taxonomic trait is morphology of cells during exponential growth in peptone-yeast extract (PYE) medium at 25 to 30°C. Pigmentation and growth factor requirements are used as secondary traits to distinguish types within each morphological group. Other authors have expressed dissatisfaction with the heavy dependence of the taxonomy of caulobacters on morphological traits (80, 131, 134), a complaint for which I feel considerable sympathy; the taxonomy of this group would be greatly advanced by further physiological characterization, particularly of nutritional properties.

Inspection of Table 1 suggests that of any 50 caulobacter isolates obtained by methods described in Enrichment and Isolation, 23 would be bacteroid, 15 would be vibrioid, 6 would be subvibrioid, 4 would be fusiform, and 2 would be *Asticcacaulis*. Krasil'nikov and Belyaev did not encounter any *Asticcacaulis* isolates from soil or water, and marine forms have not been reported. *Asticcacaulis biprosthecum* has apparently been isolated only once (213, 215); "AC-2" (178) was the label I used for the subculture received from J. L. Pate. It is also apparent that among vibrioid caulobacters, *C. vibrioides* and *Caulobacter henricii* appear most often; *C. crescentus* is not commonly encountered; of the 160 reported isolates of nonmarine *Caulobacter* types, only 14 are able to grow on glucose-mineral medium without vitamins or amino acids. All six *Asticcacaulis* isolates require biotin.

In an evaluation of DNA similarity among several caulobacter species, the only significant homology (greater than 58%) found was between

strains of *C. vibrioides* and *C. crescentus* (185). This could mean that these two species are redundant or simply that they are the only closely related subgroups among the caulobacters. No significant homology was detected between these two species and *C. henricii*, discouraging the taxonomic simplification suggested by Krasil'nikov and Belyaev (131) that only two subgeneric groups need be recognized, viz., vibrioid and bacteroid.

At present, too little additional information has accumulated to permit reevaluation of the system presently contained in *Bergey's Manual of Determinative Bacteriology* (219, 220). Studies with the caulophages (see Caulophages) have seemed to justify the primary divisions in the present taxonomic scheme, and the wide host ranges of certain caulophages that are nevertheless restricted to caulobacters have demonstrated that this is a relatively isolated bacterial group. The growing awareness of the ubiquity of caulobacters in natural and man-made bodies of water, in soils, and in algal systems and of their possible roles in the degradation of industrial and commercial contaminants of waters (187, 266) is evidence that the existing system is probably inadequate to accommodate the variety of caulobacters already encountered and maintained in collections.

## CAULOPHAGES

### Isolation of Phages Lytic for Caulobacters

The first reported isolation of a caulophage was presented in 1963 by Khavina and Rautenstein (127). One phage was isolated from a sediment sample by enrichment on their strain 76. Attempted isolations from soil samples were not successful. Schmidt and Stainer (238) isolated phages, using the CB and AC strains (218) as hosts. Direct isolation did not prove successful, but 23 phages were isolated by host enrichment from sewage and pond water, and 1 was isolated as a "contaminant" of a strain not sensitive to the phage. Soil samples, again, did not yield caulophages.

The 23 phages of Schmidt and Stanier (238) were classified in seven groups. Three of the groups comprised large, two-stranded DNA phages with prolate cylindrical heads approximately 50 by 170 nm and long, flexible, noncontractile tails 10 by 200 to 250 nm. This type is the most frequently isolated *Caulobacter* phage and is also found among *Asticcacaulis* phages (178). A second set of three groups (238) comprised small isodiametric RNA phages, approximately 23 nm in diameter. RNA caulophages have been encountered in other successful isolations of caulophages for *Caulobacter* spp. (112, 180), although none have been found for *Asticcacaulis* spp. The third type of phage was lytic only for *Asticcacaulis* spp. (238). These two-stranded DNA phages possessed icosahedral heads 65 by 70 nm and tails 150 nm long. This type of phage has been isolated from a natural sample only once on *Caulobacter* (112). However, it was the predominant type among phages isolated from sewage and lake water on *A. excentricus* by Middleton and Pate (178). The lysogenic phages obtained by Driggers and Schmidt (54) were morphologically similar to the most prevalent *A. excentricus* type, but were temperate and able to infect *Caulobacter* spp.

The second large collection of caulophages was reported by G. H. Szeyko and V. F. Gerencser (Bacteriol. Proc., p. 27, 1967). Apparently, their 34 isolates (all DNA phages) were readily distinguishable from one other by host range, since they classified them into 31 host range groups. Details regarding these phages have not been published, but some were used in a phage-typing survey (10).

Two collections of phages for *C. crescentus* have been reported (112, 180). Miyakawa et al. (180) isolated 13 phages from sewage and river water in Japan by plating water samples on *C. crescentus* CB13 without an enrichment step. (*C. crescentus* CB13 is also known as CB13B1a as a consequence of three successive reisolations of the wild type from spontaneous mucoid colony variants [Poindexter, unpublished data]. Since 1967, only CB13B1a has been available, but I have used here the designation as given in each report reviewed.) Six of the phages were RNA phages similar to Schmidt and Stanier's isolates. Two DNA phages of known designation, $\phi$C1 and $\phi$Cp34, were probably obtained in this isolation (72, 74); they are of the predominant *Caulobacter* type of phage. Johnson et al. (112), using strains CB13 and CB15 as initial hosts, isolated 38 phages, with and without enrichment, from ponds, streams, and tropical fish tanks. Most of their isolates (28 of the 38) were the large cylindrical DNA type; only 2 were RNA phages. The remaining eight isolates were morphologically varied (see Table 2).

Reports of the isolation of one or a few phages have appeared, and one of these isolates ($\phi$CbK) has been used in numerous studies. Its isolation was reported, but not described, by Agabian-Keshishian and Shapiro (4); because it is lytic only for *C. crescentus* strains (282), it presumably was isolated on CB13, CB15, KA2, or KA3. Its morphology is similar to that of the predominant type isolated by other workers (112, 238). One unusual *C. crescentus* phage, $\phi$Cd1, isolated from sewage on and lytic only for strain CB13B1a, was reported by West et al. (282). This small icosahedral phage, 60 nm in diameter, possesses a very short tail (10 to 12 nm). Only

two other such phages, φCr40 and φCr41, have been isolated for caulobacters (112).

In all, 132 caulophages have been reported; 119 are listed in Tables 2 and 3. In addition to those listed, 13 have been mentioned with little description: a temperate phage, LC72 (28); a "φCbK-like" phage (282); phage φCFD-1 (178); and 4 RNA phages in addition to φCp2 and φCp18, and 7 DNA phages, presumably including φCp34 (72, 74, 180). No caulophages containing single-stranded DNA or two-stranded RNA have been reported; none of them are known to contain lipid, and none are filamentous. Physical characterization has been reported for five of the caulophages: three of the RNA phages (16, 67, 154, 180; B. R. Neufeld, Ph.D. thesis, Indiana University, Bloomington, 1968; see also reference 250), one of the large cylindrical DNA phages (4, 148, 149, 162, 163), and the small DNA phage φCd1 (282). The morphological variety of caulophages is summarized in Table 2.

## Host Ranges on Wild-Type Hosts

Seven of the reports on caulophages have included determinations of host ranges with strains representing three or more species groups of caulobacters (10, 54, 72, 178, 180, 238, 282). Their results are summarized in Table 3. As far as can be determined, the positive reactions presented in the table represent occurrences of productive infections, with the possible exception of the susceptibility of *A. biprosthecum* to morphological type III Ac phages. Schmidt and Stanier (238) reported that infection occurred, but Middleton and Pate (178) found only inhibition of growth, not plaque formation.

Growth inhibition by phage-containing lysates is not uncommon in these systems (10, 54, 112, 178). However, in only two host-phage pairs has the basis for this phenomenon been explored. In each of those instances, phage adsorption occurred, and apparently also phage DNA penetration; the cell subsequently became leaky or lysed, and in either case died without propagating the phage. Such abortive infection has been found in phage φ6 infection of *C. vibrioides* CV-113 (114) and in phage φCp34 infection of *C. crescentus* CB1 (72); whether it is significant that these two caulophages can attach to flagella (72, 117) cannot be decided until other possible abortive caulophage infections are investigated.

Certain of the host ranges summarized in Table 3 are broad, but none is known to extend to all seven morphological host groups, nor even to all five *Caulobacter* groups. However, each of the seven host groups is lysed by at least one group of type I phages, and the majority of phage isolates in this group exhibit wide host ranges, extending to as many as four host groups. One of these phages, φCb13, is lytic for 30 of 102 *Caulobacter* strains tested (10, 238). Colorless vibrioid caulobacters can be lysed by phages of all seven types (10, 112, 238), and every one of the morphological type I phages isolated on *Caulobacter* strains can infect colorless vibrioid caulobacters, whether the phage is isolated on a host of that group or on a bacteroid strain.

Only two type II phages have been isolated on *Caulobacter*. The host range of one of these, φ6, is as broad as those seen for type I phages. The host range of the other (φ76) has not been reported in a manner allowing its incorporation into the table; it was lytic for 2 of the 10 strains tested (127).

Only two type III phages have been isolated on *Caulobacter* other than those induced in

TABLE 2. *Morphological variety among caulophages*

| Type[a] | Head | Tail | No. of isolates | Examples[b] |
|---|---|---|---|---|
| I | Prolate cylinder, 50–65 by 170–260 nm | Flexible, noncontractile, 200–320 nm long | 46 | φCr2 group of 28 isolates, $\phi AcS_3$ |
| II | Elongated polyhedron, 65–70 by 100–105 nm | Flexible, noncontractile, 260–300 nm long | 3 | φ76 |
| III | Icosahedron, 50–80 nm in diameter | Flexible, noncontractile, 150–200 nm long | 25 | φCr1, φCr22 |
| IV | Icosahedron, 80 nm in diameter | Contractile, 140 nm long | 2 | φCr30, φCr35 |
| V | Icosahedron, 140 nm in diameter | Contractile, 140 nm long | 2 | φCr24, φCr26 |
| VI | Icosahedron, 60 nm in diameter | Rigid, absent from some particles, 10 or 50–60 nm long | 3 | φCr40, φCr41 |
| VII | Isodiametric polyhedron, 20–29 nm in diameter | Absent | 12 | φCr14, φCr28 |

[a] These are not the designations proposed by Bradley (21); the most prevalent type among the caulophages (type I) was not included in his classification. Types I through VI are DNA (two-stranded) phages; type VII phages are RNA (single-stranded) phages.

[b] φ76 was isolated by Khavina and Rautenstein (127), $\phi AcS_3$ was isolated by Middleton and Pate (178), and the others were isolated by Johnson et al. (112). Additional isolates are listed by morphological type in Table 3.

TABLE 3. *Host ranges of caulophages among wild-type strains*

| Phage morphology type[a] | Reference | Phage isolates | No. of susceptible strains/no. of strains tested[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *Caulobacter* | | | | | *Asticcacaulis* | | Total |
| | | | Vibrioid | | Subvibrioid | Fusiform | Bacteroid | Excentral | Lateral | |
| | | | Colorless | Pigmented | | | | | | |
| I | 238 | φCb6, 7, 10, 11, 19 | **4/5** | 0/8 | **2/2** | **2/5** | **6/10**[c] | 0/4 | 0/1 | 14/45 |
| | 238 | φCb1, 3 | **2/15** | 0/8 | 0/2 | **2/5** | **4/10**[c] | 0/4 | 0/1 | 8/45 |
| | 238 | φCb13, 14, 16, 17, 18 | **12/15**[c] | **4/8** | 0/2 | 0/5 | 0/10 | 0/4 | 0/1 | 16/45 |
| | 178, 214 | φAc41, 42 | 0/11 | 0/3 | 0/4 | **3/5** | **3/8** | **2/4**[c] | 0/1 | 8/36 |
| | 178, 214 | φAcS$_1$, S$_2$, S$_3$ | 0/11 | 0/3 | 0/4 | 0/5 | 0/8 | 0/4 | **1/1**[c] | 1/36 |
| | 282 | φCbK | **4/12** | 0/1 | | 0/2 | 0/3 | | | 2/18 |
| | 72 | φCp34 | **4/5**[c] | | | 0/1 | 0/1 | 0/1 | | 4/8 |
| II | 10 | φ6 | **4/31** | | **1/4** | **1/6** | **3/21** | 0/15 | | 9/77 |
| | 178 | φAcM$_2$, M$_3$, M$_4$, M$_5$ | 0/11 | 0/3 | 0/4 | 0/5 | 0/8 | 0/4 | **1/1**[c] | 1/36 |
| III | 238 | φAc20, 21, 22 | 0/15 | 0/8 | 0/2 | 0/5 | 0/10 | **2/4**[c] | **1/1** | 3/45 |
| | 178 | φAc12, 13, 15, 31, 33, 38, 39, 57 | 0/11 | 0/3 | 0/4 | 0/5 | 1/8 | **2/4**[c] | 0/1 | 3/36 |
| | 178 | φAc11, 14, 35, 36, 37, 45, 46 | 0/11 | 0/3 | 0/4 | 0/5 | 0/8 | **2/4**[c] | 0/1 | 2/36 |
| | 178 | φAc59 | 0/11 | 0/3 | 0/4 | 0/5 | 0/8 | **1/4**[c] | 0/1 | 1/36 |
| | 54 | φ101 | **1/11**[c] | **1/2** | 0/2 | | | | | 2/15 |
| | 54 | φ151 | **1/11** | **1/2**[c] | 0/2 | | | | | 2/15 |
| | 54 | φ102 | **2/11**[c] | 0/2 | 0/2 | | | | | 2/15 |
| | 54 | φ118 | 0/11 | **2/2**[c] | 0/2 | | | | | 2/15 |
| VI | 282 | φCd1 | **1/10**[c] | 0/1 | | 0/2 | 0/3 | | | 1/16 |
| VII | 238 | φCb8r, 9 | **1/15** | 0/8 | 0/2 | 0/5 | **2/10**[c] | 0/4 | 0/1 | 3/45 |
| | 238 | φCb2, 5, 12r, 15 | **13/15**[c] | 0/8 | 0/2 | 0/5 | 0/10 | 0/4 | 0/1 | 13/45 |
| | 238 | φCb4 | **7/15**[c] | 0/8 | 0/2 | 0/5 | 0/10 | 0/4 | 0/1 | 7/45 |
| | 238 | φCb23r | 0/15 | 0/8 | 0/2 | **2/5**[c] | 0/10 | 0/4 | 0/1 | 2/45 |
| | 180 | φCp2, 18 | **5/5**[c] | | | 0/1 | 0/1 | 0/1 | | 5/8 |
| Unknown | 178 | Group XII (16 isolates) | 0/11 | 0/3 | 0/4 | 0/5 | 0/8 | 0/4 | **1/1**[c] | 1/36 |
| | 10 | φ26 | **18/31** | | 0/4 | **1/6** | 0/21 | 0/15 | | 19/77 |
| | 10 | φ43A, 43B, 47 | **22/31** | | 0/4 | **1/6** | 0/21 | 0/15 | | 23/77 |
| | 10 | φ36C, 36D, 36E | **2/31** | | 0/4 | 0/6 | 0/21 | 0/15 | | 2/77 |
| | 10 | φ36B | **1/31** | | 0/4 | 0/6 | 0/21 | 0/15 | | 1/77 |

[a] See Table 2.

[b] Host strains tested were CB, KA, and AC strains from the collection of Poindexter (218), Cv strains from the collection of Babinchak and Gerencser (Bacteriol. Proc., p. 28, 1969), and the two *Asticcacaulis* isolates of Pate and Ordal (213). Boldface type indicates phage susceptibility in a host group.

[c] The host strain used in the initial enrichment or isolation of the phage is in this host group.

lysogenic strains (54); the breadths of their host ranges have not been examined. In contrast, this type accounts for almost all of the phages isolated on *A. excentricus* (all 3 isolated by Schmidt and Stanier [238] and 16 of the 18 isolated by Middleton and Pate [178]). Their host range is restricted to this species, but does not extend to all *A. excentricus* strains tested; neither group of Ac phage isolates (178, 238) contained phages lytic for strains AC12 and KA4.

Host ranges of type IV and type V phages have not yet been reported. The single type VI phage for which the host range was determined, φCd1, was found restricted to the *C. crescentus* strain (CB13) on which it was isolated (282).

Among the RNA phages (type VII), there are two kinds of host ranges. One group (φCb8r and φCb9), isolated on and lytic for bacteroid caulobacters, can lyse one colorless vibrioid strain; the host range is relatively broad with respect to type of host, but only 3 of 45 strains tested were susceptible. Each of the remaining four type VII groups is restricted to a single kind of host, but then typically is infective for 40% or more of host strains of that type.

Among phages isolated on *A. excentricus*, the widest host ranges are (as in *Caulobacter*) exhibited by the type I phages. Phages φAc41 and φAc42 lyse not only *A. excentricus*, but also six *Caulobacter* strains, among them two (CB9 and CB28) for which none of the Cb phage isolates is lytic (178). Each of the phages isolated on *A. biprosthecum* is restricted to that strain alone.

To date, no caulobacter isolate has been found susceptible to any phage isolated on any other group of bacteria, nor have any caulophages been found capable of lysing bacteria of any other family (178, 218, 238, 282). Whereas the two genera of caulobacters remain distinct among eubacteria with respect to phage sensitivity, they are not isolated from each other in this respect. The intrafamily taxonomic implications are not strong, however, since intergeneric phage sensitivities are tolerated in other eubacterial groups (see, e.g., reference 164) and shared phage sensitivity cannot be used as evidence of either species or generic identity of the hosts. Among *Caulobacter* species, however, it does seem significant that many caulophages are lytic for a variety of vibrioid types—*C. vibrioides*, *C. crescentus*, and in some cases *C. henricii*—whereas others are lytic for clusters of bacteroid and fusiform types. It is doubtful that there is any cross-susceptibility between the two species of *Asticcacaulis*.

## Cross-Resistance in Phage-Resistant Variants

Phage-resistant mutants have been obtained for purposes of studying phage infections, cellular differentiation, and genetic transfer in caulobacters. Phage susceptibility has also been found altered in morphogenetic mutants, and this has usually been interpreted as evidence of pleiotropic genes governing the morphogenetic events that occur at the nonstalked pole. Objection has been raised (63, 109) to the use of mutagenesis in the isolation of *C. crescentus* mutants on the grounds that such mutagens as ultraviolet and nitrosoguanidine are known to cause multiple mutations. However, in practically all reports, cross-resistances have been studied either with mutants obtained without mutagenesis or with mutants from which spontaneous revertants have been obtained and characterized. There has not been a significant difference in cross-resistance patterns found among spontaneous and induced mutants, although the frequency of nonmotility is higher among mutagen-treated, phage-resistant mutants (33/107 [73] and 4/50 [63]) than among nonmutagenized populations (10/117 [147] and 0/150 [63]).

**Spontaneous mutants exhibiting phage resistance.** Lagenaur et al. (147) isolated spontaneous mutants resistant to phage φCbK or to phage φCb5 in order to explore the relationship among pili, flagella, and φCbK adsorption. Mutants of *C. crescentus* CB13B1a were selected as survivors of incubation with either phage; each isolate was then examined for susceptibility to each phage and for motility. Some of the results are summarized in Table 4. Every mutant exhibited some degree of reduced susceptibility to the phage that was not present during selection. Full resistance to φCbK, after selection with either phage, was accompanied by full resistance to φCb5; in contrast, full resistance to φCb5, although usually accompanied by full resistance to φCbK, occurred with only partial resistance to φCbK in 4 of the 117 mutants. Almost all the mutants were motile, and although they synthesized pilin, none assembled external pili.

Johnson et al. (112) used 12 phages in selecting resistant derivatives of *C. crescentus* CB15 that were tested for susceptibility to each of 36 phages. The results (Table 4) demonstrated that not 1 of the 12 selecting phages yielded a resistant clone with wild-type susceptibility to all the other phages. In some cases, this may reflect close relationships within each of the phage groups, but certain aspects of the resistance patterns are especially informative. First, resistance to the large DNA phages again coincided with resistance to the RNA phages. Among the phages of this group, some attach to the nonstalked pole, two (φCr14 and φCr28) attach to pili, and one (φCr26) shows no such preferential attachment site. Accordingly, resistance to this heterogeneous group cannot be accounted for solely by modifications of the nonstalked pole

TABLE 4. *Cross-resistance among host strains selected as survivors of phage infection without mutagenesis*

| Host strains from: | Phage(s) used in selection | No. of strains resistant to: | | | | | | | | | | | No. of strains cross-resistant/no. of strains tested |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Selecting phage(s) only | Additional phages[a] | | | | | | | | | | |
| | | | φCbK, (φCb5) | (φCbK), φCb5 | (φCbK, φCb5) | φCbK, φCb5 | Group 1[b] | φCr22 | Group 2[c] | φCb12r | φCb13 | φAcM$_1$, φAcML$_2$, φAcS$_2$ | |
| *C. crescentus* CB13 (147) | φCbK | 0 | 0 | 13 | 0 | 47 | | | | | | | 60/60 |
| | φCb5 | 0 | 0 | 4 | 17 | 36 | | | | | | | 57/57 |
| *C. crescentus* CB15 (112) | φCr22, 25, or 36 | 0 | | | | | 2 | 2 | 2 | | | | 2/2 |
| | φCr5, 8, 14, 21, 31, or 42 | 0 | | | | | 9 | 9 | 0 | | | | 9/9 |
| | φCr8, 30, or 33 | 0 | | | | | 4 | 0 | 4 | | | | 4/4 |
| | φCr22 | 0 | | | | | 0 | 1 | 1 | | | | 1/1 |
| | φCr30 or 41 | 0 | | | | | 0 | 0 | 4 | | | | 4/4 |
| | None | | | | | | 0 | 1 | 0 | | | | 0/1 |
| *C. crescentus* CB15 (63) | φCbK | 1 | | | | | | | | 149 | 149 | | 149/150 |
| *A. biprosthecum* (214, 279) | φAcM$_1$ | 0 | | | | | | | | | | 1 | 1/1 |
| | φAcML$_2$ | 0 | | | | | | | | | | 1 | 1/1 |
| | φAcS$_2$ | 0 | | | | | | | | | | 54 | 54/54 |

[a] Parentheses indicate reduced susceptibility.

[b] Twenty-eight type I (DNA) phages (including φCr5, φCr8, φCr21, φCr25, φCr31, φCr33, φCr36, and φCr42), two type VII (RNA) phages (φCr14 and φCr28), and φCr26.

[c] DNA phages φCr30, φCr35, φCr40, and φCr41.

and its pili. Further, resistance was invariably accompanied by resistance to phage φCr22 or the second group of phages or both.

In contrast, resistance to the second group of phages could be selected unaccompanied by resistance to phages outside this group. It is possible that these phages, all of which attach at random sites on the cell, depend on the presence of receptor sites that are more specific than those involved in infection by the majority of caulophages. Relative frequencies of mutants obtained by the various selections were not reported.

Selection of *A. biprosthecum* mutants by phage infection also results in cross-resistance (Table 4).

**Phage resistance subsequent to mutagenesis.** Fukuda et al. (73, 74) reported observations with a group of mutants derived from nitrosoguanidine-treated *C. crescentus* CB13B1a; survivors of phage infection with phage φCp2, φCp34, or φCbK were isolated. The resistance patterns of these mutants are presented in Table 5. As generally observed (110, 112, 147), resistance to phage φCbK, whether selected or unselected, was in all cases accompanied by resistance to all the other phages. Among the 107 mutants were 33 that had become nonmotile. Five spontaneous motile revertants of nonmotile mutants resistant to all three phages exhibited full wild-type phage susceptibilities, but three retained resistance to phage φCp2, indicating that at least some of the mutants in this study harbored more than one mutation.

In an attempt to assess the influence of nitrosoguanidine mutagenesis on the frequency of pleiotropism in phage φCbK-selected mutants, Farrell and Smith (63) isolated, from *C. crescentus* CB15, 150 nonmutagenized resistant mutants (Table 4) and 50 mutants treated with nitrosoguanidine before exposure to the phage (Table 5). All but 1 of the 200 mutants became resistant also to phages φCb12r and φCb13 (238; see Table 3). In both groups, about one-third of the mutants exhibited reduced motility, but only among the nitrosoguanidine-treated survivors were any (four) nonmotile phage-resistant strains found. Of 51 motile revertants isolated from the four strains, 20 regained susceptibilities to all three phages; the others remained fully phage resistant. Again, partial reversion implied that multiple mutations had occurred.

It should be noted in particular that, with the exception of phage φCp34, lost or reduced adsorption capacity for unrelated phages is typical of mutants resistant to type I phages (73, 109, 112, 147, 214), the wide-host-range caulophages (see Table 3). The affected phages include RNA phages, known to be serologically distinct from type I phages (180, 238), pole-specific DNA phages, and random-attachment-site phages. This implies that a process related to assembly, localization, or function of cell surface components in general, rather than primary structure of specific phage receptors, is a common factor important in the adsorption of most of the caulophages (73, 112, 237, 240).

## Lysogeny and Transduction

Attempts to detect classical lysogeny in caulobacters have generally been unsuccessful (112, 178, 238). However, Johnson et al. (112) stated that 20 of their type I phages were capable of establishing stable lysogenic relationships with their hosts; details were not presented.

The one detailed report of lysogeny in caulobacters is the study by Driggers and Schmidt (54). In an extension of the earlier study of Schmidt and Stanier (238), in which attempts to induce multiplication of lysogenic phages by ultraviolet were not successful, 19 *Caulobacter* and 2 *Asticcacaulis* strains were examined for inducibility by mitomycin C and ultraviolet. Six strains responded to each inducer by producing phagelike particles; indicators were found for

TABLE 5. *Cross-resistance among host strains selected as survivors of phage infection after nitrosoguanidine mutagenesis*

| Host strains derived from: | Phage used in selection | No. of strains resistant to: | | | | | | | No. of strains cross-resistant/no. of strains tested |
|---|---|---|---|---|---|---|---|---|---|
| | | Selecting phage only | Additional phages | | | | | | |
| | | | φCp2, 18 only | φCp1, 34 only | φCbK only | φCp1, 2, 18, 34, φCbK | φCb12 | φCb13 | |
| *C. crescentus* CB13 (70, 73, 74) | φCp2 | 0 | 4 | 12 | 0 | 34 | | | 50/50 |
| | φCp34 | 3 | 4 | 0 | 0 | 6 | | | 10/13 |
| | φCbK | 0 | 0 | 0 | 0 | 44 | | | 44/44 |
| *C. crescentus* CB15 (63) | φCbK | 0 | | | | | 50 | 50 | 50/50 |

four of these phages. Each lysogenic strain produced a phage distinguishable from the others by host range, was resistant to lysis by the phage it produced, and could not be cured of inducibility by repeated exposure to specific antiphage serum. All four phages were morphological type III. The two phages produced by *C. vibrioides* strains were serologically related, but did not cross-react with the two phages produced by *C. crescentus* strains; the latter two phages were not serologically related to each other.

Use of the lysogenic phages in transduction attempts has not been reported. However, two phages (φCr30 and φCr35 [59, 110, 112]) virulent for *C. crescentus* CB15 have been found capable of generalized transduction. Their ability to transduce was interpreted (59) as being a consequence of their slow adsorption and prolonged latent phase, allowing formation of transductants that escaped lytic infection. Nevertheless, ultraviolet irradiation of phage lysates to inactivate at least 99.999% of the plaque-forming units was necessary to prevent phage multiplication in recipient populations.

When used by Ely and Johnson (59) in analysis of auxotrophic mutants, transduction as mediated by phage φCr30 appeared generalized. Six auxotrophic mutants exhibiting five different amino acid auxotrophies were able to act as recipients; prototrophic transductants in excess of spontaneous revertants resulted from exposure of each mutant to irradiated phage grown on the parental strain, CB15. The authors noted, however, that whereas phage φCr30 appeared able to transduce markers from several regions of the CB15 genome, it yielded transductants at different frequencies for different markers; frequencies varied up to 8-fold with different markers and up to 400-fold with multiplicity of infection. Although not restricted to one or a few loci, phage φCr30 nevertheless exhibited wide variation in affinity for different genes. Affinities for auxotrophic markers seemed more variable than those for motility genes (cf. references 59 and 110).

Such a system is difficult to use for primary mapping of a genome whose geography has not been established by an independent type of analysis. The mapping of phage φCr30 affinity regions will greatly increase the usefulness of the transduction system. Nevertheless, by normalizing transductant frequencies to "expected values" based on transducibility of a locus as determined with a wild-type donor, it is possible to detect nonlinkage and to estimate linkage. Major problems inherent in using such a system are that genes located in low-affinity regions may appear misleadingly to fall into "tightly linked clusters" (59) or may be transduced at frequencies so low that they approach reversion frequencies (58, 110). An additional problem with the phage φCr30 system in particular is that variation in susceptibility to the phage may occur as a consequence of mutagenesis (109, 112) or of selection by survival of infection with other phages (112). Particularly in the latter case, the suitability of phage φCr30-mediated transduction may be limited in the analyses of phage resistance mutations and of mutations affecting polar morphogenesis, many of which are pleiotropic (see Table 7).

Despite these problems, phages φCr30 and φCr35 are the only transducing caulophages yet available, and they have been used in genetic analyses (33, 59, 60, 68, 110). Close linkage of glycerol auxotrophy genes was inferred from relative transductant frequencies of only 2 and 3% (34). In a semiquantitative analysis of nonmotile mutants (110), *fla* mutations were classified into 26 linkage groups; *mot* (paralyzed flagella) mutations did not appear linked to one another. Two of the glutamate synthase mutations appeared linked (58); the other two mutations were not linked to these and not clearly linked to each other, since relative transductant frequencies depended on which strain served as donor.

### Summary

As a group, the caulophages exhibit at least seven distinctive morphologies of head and tail, including a majority with noncontractile tails, and both virulent and temperate infection cycles. The predominant morphological type exhibits the widest host range, and mutation to resistance to phages of this type is typically accompanied by total phage resistance in the host. Continued studies of resistance to this type of caulophage in particular may elucidate major aspects of surface morphogenesis in the caulobacters.

To date, all of the caulophages from natural samples have been obtained from water, but, in contrast to their hosts, they have frequently been isolated from sewage and eutrophic waters.

## CULTIVATION AND NUTRITION

### Laboratory Cultivation

The strains of caulobacters that have been isolated to date are all aerobic, low-mesothermal chemoheterotrophs. The great majority of isolates require one or more organic growth factors; for a few such strains, growth in defined media is supported by one or more B vitamins (20, 134, 152, 218), but for many isolates the requirements

available in peptone or yeast extract remain undetermined.

**Media.** For general cultivation, a Mg-supplemented (0.01 to 0.02% $MgSO_4 \cdot 7H_2O$) peptone (0.2%)-yeast extract (0.1%) (PYE) medium prepared in tap water (218) or distilled water (5, 249, 280) has proved suitable. Some workers have found additional Ca (0.5 mM) supplementation useful (109). For the past several years, the PYE medium used in my laboratory has been prepared in distilled water supplemented with vitamin-free Hutner base (32) rather than $MgSO_4$ alone (185, 224). This practice, also used in J. M. Schmidt's laboratory (26, 29, 54, 119) and occasionally by others (180), has reduced variation in mineral content of the medium and improved reproducibility of both rate and yield of growth with all strains used. Workers in the Soviet Union have used either PYE (14, 130, 131) or similar dilute media, usually containing peptone, with or without yeast extract (12, 13, 14, 80), at total organic concentrations ranging from 0.05 to 0.3%.

For defined media (Table 6), the most widely used basal solution has been Hutner mineral base (32) prepared without vitamins, with phosphate as the pH buffer (218) and designated in this review "Hp." With glucose as sole carbon source, this medium, variously designated "Hmg," "GMS," or "M3," has served as the defined medium in almost all of the studies reported from the laboratories of L. Shapiro

TABLE 6. *Basal solutions used in defined media for caulobacters*[a]

| Basal solution | Composition | Species cultivated[b] | Reference |
|---|---|---|---|
| Hp (Hutner mineral base with phosphate buffer) | 20 mM sodium phosphate and potassium phosphate<br>9.3 mM $NH_4Cl$<br>Hutner base[c] | *C. crescentus*<br>*C. vibrioides* ($B_2$)<br>*C. henricii* ($B_{12}$)<br>*C. leidyi*<br>*A. excentricus* (biotin) | 218 |
| Hi (Hutner mineral base with imidazole buffer) | 0.1-1 mM sodium phosphate and potassium phosphate<br>9.3 mM $NH_4Cl$<br>5 mM imidazole<br>Hutner base | *C. crescentus*<br>*A. excentricus* (biotin) | 239 |
| M2 | 20 mM sodium phosphate and potassium phosphate<br>9.3 mM $NH_4Cl$<br>0.5 mM $MgSO_4$<br>0.5 mM $CaCl_2$<br>0.01 mM $FeSO_4$<br>0.008 mM ethylenediaminetetraacetic acid | *C. crescentus* | 109 |
| M2, modified | 2 mM sodium phosphate and potassium phosphate<br>9.3 mM $NH_4Cl$<br>0.5 mM $MgSO_4$<br>0.5 mM $CaCl_2$<br>0.01 mM $FeSO_4$<br>0.008 mM ethylenediaminetetraacetic acid<br>20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid | *C. crescentus* | 35 |
| Minimal | 7.6 mM potassium phosphate<br>7.6 mM $(NH_4)_2SO_4$<br>0.4 mM $MgSO_4$ | *C. vibrioides* ($B_2$)<br>*C. crescentus* | 115 |
| MS-B | 1 mM sodium phosphate and potassium phosphate<br>0.4 mM $MgSO_4$<br>0.4 mM sodium citrate<br>Trace salts[d] | *A. excentricus* (biotin)<br>*A. biprosthecum* (biotin) | 152 |

[a] Each medium is adjusted to pH 6.8 to 7.2. Glucose, 0.2% (11 mM), is suitable as the carbon source in all media; the suitabilities of other sugars depend on the strain to be cultivated.

[b] Required vitamin supplements are in parentheses.

[c] Hutner base (32), prepared without vitamins; nitrilotriacetic acid and ethylenediaminetetraacetic acid as chelating agents; salts ($MgSO_4$ and $CaCl_2$); and trace salts [$Co(NO_3)_2$, $CuSO_4$, $FeSO_4$, $MnSO_4$, $Na_2B_4O_7$, $(NH_4)_6MO_7O_{24}$, and $ZnSO_4$].

[d] Trace salts: $CaCl_2$, $CoCl_2$, $CuSO_4$, $FeSO_4$, $K_2B_4O_7$, $MnSO_4$, $MoO_3$, $ZnSO_4$. Nitrogen provided as $(NH_4)_2HPO_4$ or amino acid.

(136, 137, 140, 172, 249, 251), N. Agabian (1, 3, 60, 61, 140, 143, 145, 147), A. Fukuda (73, 74, 99, 101), R. G. Riley and B. J. Kolodziej (227), and A. Newton (27, 47, 48, 193, 194, 205–207, 209, 254, 276, 277).

The inorganic phosphate concentration in Hp basal medium is 20 mM. Schmidt and Stanier (239) found that 0.1 mM phosphate was sufficient to support maximal growth with 0.2% glucose in an imidazole-buffered medium (G-I; designated "Hi" in Table 6). This medium, with phosphate at 0.1 to 1 mM, has been used not only in Schmidt's laboratory (83, 233, 234, 237, 239), but also by Newton's group (199, 200, 206, 207, 253), Shedlarski (252), and me (221, 222).

A third basal medium was designed in the laboratory of B. Ely (109; see also references 58, 59, and 110). Designated "$M_2$," the medium contains 20 mM phosphate as the pH buffer and substitutes Mg, Ca, and Fe salts chelated with ethylenediaminetetraacetic acid for the more elaborate mineral base of Hutner. The high concentration of phosphate may be sufficient to carry, as chelated contaminating metals, other trace metals required for growth. Neidhardt et al. (189) have shown that the phosphate concentrations of media for enteric bacteria, usually 70 to 150 mM, can be reduced to 1.32 mM when specific trace metals are provided. Jollick and his co-workers (6, 115, 116) used a defined medium ("minimal" in Table 6) containing phosphate (7.6 mM), ammonium, and Mg salts, but no further added minerals. They did not mention cultivation in liquid medium; the trace minerals contained in commercial agar may have been sufficient to support growth on their plating medium.

Both Hp and Hi with 0.2% glucose, supplemented with biotin, are suitable media for the cultivation of *A. excentricus* (218, 239). However, Larson and Pate (152) found that the chelating agents present in Hutner base were toxic for *A. biprosthecum*. In their "MS-B" medium (Table 6), sodium citrate served as the chelating agent for the trace metals provided. *A. excentricus* did not exhibit any sensitivity to glucose up to 10 mM (0.18%), the concentration typically used in the minimal media. However, the growth rate of *A. biprosthecum* showed a sharp maximum at 0.6 mM glucose; at lower glucose concentrations, morphology was uniform, but at higher concentrations, cells became highly pleomorphic. Concentrations of $(NH_4)_2HPO_4$ greater than 0.075 mM had a similar adverse effect on growth rate and normal, uniform cellular development of *A. biprosthecum*.

**Organic micronutrients.** In the earlier study of aquatic isolates (218), caulobacters that did not grow in Hp-glucose medium were found unresponsive to amino acid supplements. Krasil'nikov and Belyaev (132) reported the same observation with their collection of isolates obtained predominantly from soil. Caulobacters whose growth is supported or stimulated by exogenous vitamins respond to a narrow group of B vitamins. When a single vitamin is sufficient to support growth in glucose minimal medium, it is biotin, cyanocobalamin ($B_{12}$), or riboflavin ($B_2$) (6, 20, 132, 152, 218). Several isolates require both vitamin $B_2$ and vitamin $B_{12}$ (132). None of the isolates tested responded to nicotinic acid, pantothenic acid, $\rho$-aminobenzoic acid, thiamine, or pyridoxine (132, 218). However, the majority of strains tested did not respond to any vitamin supplementation, and it remains possible that "fastidious" caulobacters cannot generally use $NH_3$ as a nitrogen source. Alternatively, since all screening so far has used Hp-glucose medium, the excessive phosphate (20 mM) may have prevented growth responses to the vitamins or to the amino acids.

One consequence of the lack of defined media for the majority of caulobacter isolates has been to focus most studies on *C. crescentus* and, to a lesser extent, on *Asticcacaulis* species. This has resulted in investigation of one species, *C. crescentus*, in relative depth, and such focus is valuable. However, it could lead to disregard of the fact that *C. crescentus*, by virtue of its nutritional independence and relatively rapid growth rate (the very reasons for its wider experimental use), seems physiologically atypical among *Caulobacter* species. There is a clear need for more adequate physiological characterization of other species to support interpretations of both environmental and endogenous influences on morphogenesis and of the ecological role of these stalked bacteria.

**Mineral nutrients: phosphate.** The mineral nutrient so far found to have the most profound influence on growth and morphology of caulobacters is orthophosphate. Grula et al. (82) found that the minimal concentration required to support growth of *C. vibrioides* (ATCC 11764; "CB-G" in reference 218) was 25 mg/liter (0.18 mM) and that optimal growth occurred over the wide range 125 to 2,000 mg/liter (0.9 to 14.3 mM). The effect of concentrations greater than 14 mM was not described. Schmidt and Stanier (239) reported that the growth yields of *C. crescentus* CB15 and of *A. excentricus* AC48 in Hi medium with 0.2% glucose were limited by phosphate concentrations lower than 0.1 mM. In comparison with cells grown in 1 mM phosphate, 0.01 mM phosphate-grown cells of *C. crescentus* were slightly longer and possessed (by stationary phase): stalks that were several times longer, more elaborate internal mem-

branes, significantly lower contents of nucleic acids and phospholipid phosphorus, somewhat less protein, and 2.5 times as much poly-β-hydroxybutyrate.

In reexamination of the effects of phosphate limitation on growth and morphology, it has been found that, in Hi medium with 0.2% glucose, phosphate becomes growth yield limiting at 0.1 mM and also growth rate limiting at 0.02 mM for *Caulobacter leidyi* as well as for *C. crescentus* and *A. excentricus* strains (J. S. Poindexter, unpublished data). All three species exhibit significant stalk elongation relative to cell elongation when growth is phosphate limited, a phenomenon that has been observed in other prosthecate bacteria (283). During the studies with caulobacters, it was found that some strains (notably *C. crescentus* CB2) were sensitive to phosphate concentrations equal to 1 mM; growth occurred at the maximal rate with 1 mM phosphate, but yield was reduced.

In modified M2 medium (Table 6), the phosphate concentration has been reduced from 20 to 0.2 mM and a piperazine derivative has been provided as a pH buffer. Although not discussed by Contreras et al. (35; see also reference 34), this may have been done to improve labeling of phospholipids by means of exogenous [$^{32}$P]orthophosphate.

**Mineral nutrients: metals.** The earliest systematic study of mineral requirements of a caulobacter isolate, identified as *C. vibrioides* (ATCC 11764), revealed complicated interactions among the metal salts tested (82). In the presence of an optimal concentration of $FeSO_4$, no further mineral requirements were detectable. At suboptimal iron concentrations, $CaCl_2$ and $Na_2S$ were stimulatory, and Mn (possibly also Zn and Cu) appeared toxic. For soil isolates, Belyaev (14) reported that $Mg^{2+}$ and $Ca^{2+}$ were favorable for growth, whereas $K^+$ and $Na^+$ adversely affected growth; the effective concentrations were not given.

Only one study of mineral requirements in a caulobacter isolate has been reported with a full description of the methods and criteria employed. By single-factor omission screening in liquid media, followed by determination of growth rates in successive subcultures in presumptively adequate media, Larson and Pate (152) were able to infer absolute requirements of *A. biprosthecum* for salts of Ca, Cu, Co, Fe, B, Mo, Mn, and Zn. The major mineral nutrients $MgSO_4$ (0.4 mM), $KH_2PO_4$ (0.5 mM), and $Na_2HPO_4$ (0.5 mM) were present in the basal medium, which was chelated with 0.1% sodium citrate and supplemented with biotin.

At present, there is no clear characterization of the mineral requirements of any other *Caulobacter* or *Asticcacaulis* strain. There may be two major impediments to a definitive determination of the requirements. First, requirements seem to be satisifed by very low concentrations of cations, making absolute exclusion of a given metal dependent on rigorous precautions in the preparation of all media components (above all, the water [110, 139]) and vessels. Second, it seems plausible that metabolic or transport systems in these bacteria may be of such low specificity that substitutions and toxicities can result in unexplainable growth responses in the tests for mineral requirements. Both of these situations should not be unexpected in microorganisms that survive, even thrive, in such environments as stored distilled water. It may be that the most important ingredients in the widely used and generally suitable mineral mixture of Hutner (Table 6) are the chelating agents, ethylenediaminetetraacetic acid and nitrilotriacetic acid, present in the final medium at 0.009 and 1.05 mM, respectively. Their presence possibly controls the availability of the metals so that free cations are present in sufficiently low concentrations to prevent interference with cation management by the uptake systems. The principal difference between these bacteria and such groups as enteric bacteria may lie in the greater sensitivity of caulobacters to higher concentrations of salts. Whether they can accumulate mineral nutrients from relatively low environmental concentrations remains to be determined.

**Aeration.** Caulobacters are generally cultivated aerobically, and the growth rate is reduced by limiting aeration. In *C. crescentus*, $O_2$-limited cells grew as filaments with proportionally very short stalks; cell division was frequently anomalous, giving rise to siblings of unequal size (31). It was concluded that growth was dependent on oxygen respiration, since the capacity for respiratory metabolism was maximized in the $O_2$-limited cells by increased synthesis of heme proteins and elaboration of the cytoplasmic membrane in the form of small peripheral mesosomes.

*A. biprosthecum* appears to be an exception among caulobacters; its growth was markedly delayed, and yield was reduced when it was grown with vigorous aeration in MS-B (Table 6) plus glucose, maltose, galactose, or xylose plus five amino acids (152). However, growth did occur with glucose when the cultures were incubated without agitation, but open to air. Maximal growth (as rate and as yield) was obtained by allowing 24 h of static incubation, followed by aeration. In fermentor cultures with controlled levels of dissolved $O_2$, *A. biprosthecum* grew in the presence of 6.0 to 6.7 mg of $O_2$ per

liter, but not at levels greater than 6.7 mg/liter (90% saturation). Thus, *A. biprosthecum* appears to be an $O_2$-sensitive oxybiontic organism (66, 186), and its growth was not delayed due to a requirement for accumulation of $CO_2$ or any other gaseous metabolic product. Oxygen sensitivity has not been noted for any other caulobacter, including the closely related *A. excentricus*.

Many isolates have been found capable of nitrate reduction (80, 132, 175, 218), but reduction does not result in $NH_3$ or $N_2$ formation or support significant anaerobic growth. Similarly, anaerobic growth of caulobacters does not occur by means of sulfate reduction (132) or fermentation of sugars (80, 132, 218).

**Temperature.** Caulobacter cultures are routinely incubated at 30°C, less often at 28 or 33 to 34°C, and at 37°C only for special purposes. The highest incubation temperature used for the cultivation of *C. crescentus* was 42°C (172); however, 37°C has been more commonly used for studies of thermosensitive traits in both wild-type and mutant strains (137, 140, 172, 206–209, 218). Growth rate is not increased by incubation at temperatures above 30°C (140, 172, 218), as also reported for *C. vibrioides* (20), *C. bacteroides* (218), and an unidentified isolate from a stalactite grotto (175). Observations reported by Fukuda and co-workers (73, 75) revealed that phage adsorption, efficiency of plating, and sensitivity were altered in wild-type *C. crescentus* (CB13) at 35°C. In the thermosensitive *ple-801* mutant, motility (as well as phage interactions) was reduced at 35 relative to 30°C. Growth rates were not given.

The temperature characteristics of isolates from soil are essentially the same as those observed in aquatic isolates. Belyaev (14) found that growth of soil isolates was negligible at 4°C, slow at 18°C, and optimal at 28 to 30°C. Growth occurred at 37°C, but death occurred early, and 37°C-grown populations could not be subcultured.

During the maintenance of vegetative stock slants of *Caulobacter* and *Asticcacaulis* isolates, it has been found in my laboratory that morphology is more uniform, motility is higher and sustained longer, and survival during refrigeration is prolonged by incubation of the slants at 23 to 25°C rather than at 30°C. Cultures are transferred every 8 to 9 weeks, rather than at 5-week intervals as previously described (218). Stock cultures of marine isolates are cultivated at 20 to 25°C (268). Growth is somewhat slower and morphology is less uniform at 30°C for most marine caulobacters, although some isolates were reported able to grow at 37°C (161).

**pH.** The pH of minimal media generally suitable for the cultivation of caulobacters is near neutrality (Table 6). The pH of the most widely used complex medium, PYE (218), is likewise close to 7.0. Each caulobacter collection that has been surveyed, whether of freshwater, marine, or soil isolates, has exhibited the same preference for near neutrality (14, 20, 80, 160, 161, 175, 218). This is consistent with the unvarying observations that caulobacters occur in natural waters and soils within the pH range of about 5 to 9.

## Utilization of Principal Nutrients

**Carbon sources.** In the first study of C source utilization patterns among caulobacter isolates, no one compound tested was utilized by every isolate (218). In contrast, Krasil'nikov and Belyaev (132) later found that all 127 of their isolates utilized glucose, sucrose, fructose, maltose, pyruvate, alanine, glutamate, and aspartate. In further contrast between the two surveys, Krasil'nikov and Belyaev found that none of their strains utilized lactate, valine, or alcohols (methanol, ethanol, or butanol), whereas some of the earlier isolates could use these compounds, and a majority utilized butanol (218). In the study by Larson and Pate (152), *A. biprosthecum* utilized a similar group of C sources. Neither Krasil'nikov and Belyaev nor Larson and Pate tested aromatic compounds, some of which were utilized by strains in the earlier collection (218).

Studies of intermediary metabolism of carbon sources in caulobacters have been directed principally toward elucidating metabolism of glucose (152, 218, 227, 252), galactose (137), and lactose (152). All caulobacters so far tested possess glucose-6-phosphate dehydrogenase (EC 1.1.1.49). In all cases, significant constitutive activity was found, but a 2- to 13-fold increase was detected in extracts of cells grown in the presence of glucose; maximum stimulation of enzyme formation was observed in minimal medium (252). The enzyme from all strains was more active with nicotinamide adenine dinucleotide (NAD) phosphate than with NAD, demonstrable as a 10-fold difference in $K_m$ (29 $\mu$M for NAD-phosphate, 290 $\mu$M for NAD) (252). The enzyme seemed to contain separate sites for the cofactors, as evidenced by NAD-phosphate-site-specific product inhibition by reduced NAD-phosphate. Phosphoenolpyruvate and adenosine triphosphate also inhibited enzyme activity.

Detection of 2-keto-3-deoxy-6-phosphogluconate aldolase activity implied that glucose was catabolized via the Entner-Doudoroff pathway; absence of fructose-diphosphate aldolase (EC 4.1.2.13)(140, 218) and of phosphogluconate dehydrogenase (EC 1.1.1.43) (227), and the pres-

ence of 2-keto-3-deoxy-6-phosphogalactonate aldolase (137) are further evidence that the Entner-Doudoroff pathway serves as the principal means of hexose metabolism in caulobacters.

Like glucose-6-phosphate dehydrogenase, enzymes involved in metabolism of mannose and galactose exhibited moderate constitutive levels that were increased 1.5- to 5-fold in wild-type strains by growth in the presence of substrate sugars. Enzymes involved in xylose and lactose utilization exhibited relatively low constitutive levels and greater increases (12- to 46-fold) when induction resulted from growth in the presence of xylose (218) or lactose (140). *A. biprosthecum*, in contrast to *C. crescentus*, synthesized β-galactosidase (EC 3.2.1.23) constitutively and utilized glucose and lactose simultaneously (152). In *C. crescentus*, permease systems for galactose and mannose were found to be constitutive, whereas lactose uptake was increased 10-fold by growth in lactose medium (140).

**Influence of nucleotides.** Cyclic nucleotides have been examined for their influence on enzyme induction (and morphogenesis) in *C. crescentus*. The presence of cyclic nucleotides, nucleotide cyclase activity, and specific cyclic nucleotide binding proteins (249, 270, 271) and protein acyl kinase activity (2) suggests that *C. crescentus* uses this class of substances, presumably as regulatory molecules. A further rationale for such explorations has been that this class of compounds has been demonstrated to participate in regulation of inducible enzymes in bacteria (211, 212) and to influence morphogenesis relating to resting stages in myxobacteria (285), apparently as a consequence of their more immediate influence on amino acid biosynthesis (124, 170). Intracellular levels of intermediary metabolites also affect differentiation in bacilli (65).

In studies of the possible influence of cyclic nucleotides on enzyme induction in *C. crescentus*, Shapiro and co-workers (139, 140, 249) observed that when wild-type strain CB13 was shifted from Hp-glucose medium to Hp-lactose, the consequent growth lag was reduced by addition of dibutyryl cyclic adenosine monophosphate to the lactose medium. The addition of dibutyryl cyclic adenosine monophosphate resulted in earlier β-galactosidase synthesis, allowing growth at the expense of the lactose. The lag in enzyme synthesis was attributed to glucose repression; PYE-grown cells shifted to Hp-lactose exhibited a much shorter lag, and in such cells the lag was increased by the presence of glucose during lactose induction (249). However, the glucose-increased lag was not relieved by dibutyryl cyclic adenosine monophosphate. Significant fluctuation in intracellular levels of cyclic adenosine monophosphate (140, 249), adenylate cyclase (249), or cyclic guanosine monophosphate (140) were not detected during enzyme induction or during glucose repression of induction.

In certain shifts (viz., glucose to lactose, galactose, or Hp minimal medium without a sugar), but not in others (glucose to mannose, ribose, or fructose), the cell cycle was arrested throughout the population at a stage prior to flagellum activation before cell division (see Fig. 2, above). In each case, the growth lag after the shift was reduced by dibutyryl cyclic adenosine monophosphate, and any arrest in development was relieved as sugar utilization and growth proceeded.

By microscopic screening of colonies derived from survivors of ethyl methane sulfonate or ultraviolet exposure, Schmidt (233) and Schmidt and Samuelson (237) obtained mutants ("Skl") of *C. crescentus* strains CB1, CB2, and CB15 that formed long stalks (up to 20 μm) in PYE medium, in which the stalks of the parental strains were 1 to 3 μm. After initial observations that phosphate at more than 10 mM caused phenotypic reversion of stalk length in some of the Skl mutants (233), Schmidt and Samuelson (237) reported that stalks of all the mutants were of normal (wild-type) length in media containing exogenous nucleoside triphosphates. The only organic phosphate compound tested that promoted stalk elongation was cyclic guanosine monophosphate; its effect was reversed by nucleoside triphosphates, cyclic adenosine monophosphate, or dibutyryl cyclic adenosine monophosphate. The stalks of "Sei" mutants were shorter than those of the wild type in all media, but these mutants responded to phosphate by developing still shorter stalks at higher phosphate concentrations (234).

In the absence of cyclic guanosine monophosphate, excessive stalk elongation in the mutants and in phosphate-limited wild-type cells was correlated with low intracellular adenosine triphosphate pools (237). The exogenous nucleoside triphosphates increased the adenosine triphosphate levels in the mutants, but the effect of cyclic nucleotides on adenosine triphosphate levels was not reported. Wild-type cells exhibited a sharp decrease in intracellular adenosine triphosphate as the growing population entered stationary phase, at which time the wild-type stalks elongated. On the bases of these findings and preliminary studies of oxygen quotients and cytochrome content, Schmidt and Samuelson (237) ascribed the relatively low adenosine triphosphate pools of Skl strains to

deficient oxidative phosphorylation. This metabolic deficiency appeared to be the primary defect in the mutants, and their developmental abnormality appeared to be a consequence of the metabolic defect. Accordingly, morphogenetic effects of cyclic nucleotides may be related to nucleoside triphosphate levels or to adenylate charge (8, 227), which in turn must be affected not only by the exogenous phosphate concentration, but also by the rates of sugar catabolism and oxidative phosphorylation.

During transition and stationary phases in caulobacter cultures, stalk development (initiation and elongation) is the predominant morphogenetic event (77, 97, 98, 122, 152, 175, 222, 227, 237, 247), and phosphate limitation results in excessive stalk elongation in several species. Similarly, dilute nutrient environments are regularly reported to promote stalk elongation in caulobacters (see, e.g., references 20, 78, 80, 93, 106, 131, 152, 160, 161, and 291), and this could imply that stalk hypertrophy is due to depletion of the carbon source. However, in laboratory-cultivated populations in which stalk length approaches that seen in natural populations, the cells possess large accumulations of poly-$\beta$-hydroxybutyrate (222, 227, 239). Such cells seem unable to utilize available carbon for growth and so store it as lipid reserve. It is possible that a specific ratio of nutrients is necessary for carbon catabolism to proceed to exhaustion, whereas other conditions promote carbon storage. The latter conditions, rather than C source depletion, are associated with excessive stalk elongation in laboratory populations.

**Nitrogen sources.** Ammonium ion is the most suitable inorganic N source for nutritionally independent caulobacters, and they are also able to use amino acids as sources of nitrogen (see, e.g., references 82, 152, and 221). Auxotrophic mutants dependent on amino acids as the source of nitrogen have been reported (58). In their study of glutamate-dependent auxotrophs, Ely et al. (58) detected glutamate synthase and glutamine synthetase (EC 6.3.1.2) in the wild-type parent, but glutamate dehydrogenase (EC 1.4.1.2) was not detectable. They concluded that the first two enzymes served as the sole means of ammonium assimilation in *C. crescentus* CB15. The activities of both enzymes were reduced severalfold in complex medium; in defined medium (M2 with glucose), 10 mM ammonium reduced glutamine synthetase activity more than twofold relative to its activity in ammonium-limited cells. As pointed out in an earlier discussion (222), these observations would lead to the prediction that in the presence of high levels of ammonium and absence of an alternative nitrogen source (such as an amino acid), *C. crescentus* would be restricted in its ability to assimilate the available ammonium. This is so far the only concrete metabolic basis known for the seeming preference of caulobacters for dilute nutrient environments.

## CELL STRUCTURE AND COMPOSITION

The peculiar morphology of the dividing caulobacter cell is the basis for the taxonomic isolation of the genera *Caulobacter* and *Asticcacaulis* in the current "Key for the Determination of the Generic Position of Organisms Listed in the Manual" of *Bergey's Manual of Determinative Microbiology* (23). It is also the principal incitement to research in this group of bacteria, because it represents a degree of internal procaryotic differentiation for which there seems to be no anatomical explanation and because the products of cell division exhibit biosynthetic differentiation. Consequently, detailed description of the structure, composition, and function of key components, particularly those related either to surface morphogenesis or to differential gene expression, has been actively pursued during the past 2 decades. In most of the studies, at least passing attention has been given to comparative studies of the three basic cell forms of caulobacters: swarmers and dividing and nondividing stalked cells. Such studies provide the background information necessary for identification of the endogenous determinants of the developmental cycle and of the targets of exogenous influences on the occurrence and extent of differentiation.

### Fine Structure

The basic features of caulobacter fine structure are similar to those of gram-negative bacteria in general (Fig. 3). The nucleoplasm is disperse, and the cytoplasm is occupied principally by ribosomes (213, 218, 223, 279). Granules of poly-$\beta$-hydroxybutyrate are typical of cells in section, particularly when grown in minimal media or under conditions of phosphate limitation (233). The cell envelope consists of a three-layered outer membrane, a layer of peptidoglycan approximately 4 nm thick (31, 213, 218), and an inner cytoplasmic membrane.

A striking feature of caulobacter fine structure is the presence of elaborate mesosomes, known in, but not typical of, chemoheterotrophic gram-negative bacteria (79, 229). Large mesosomes are typical of the division site of caulobacters in both fixed (218, 223) and unfixed (31) cells, whether division occurs by constriction (as in *Caulobacter*) or by septation (as in *A. excentricus*). Smaller mesosomes occur at sites other

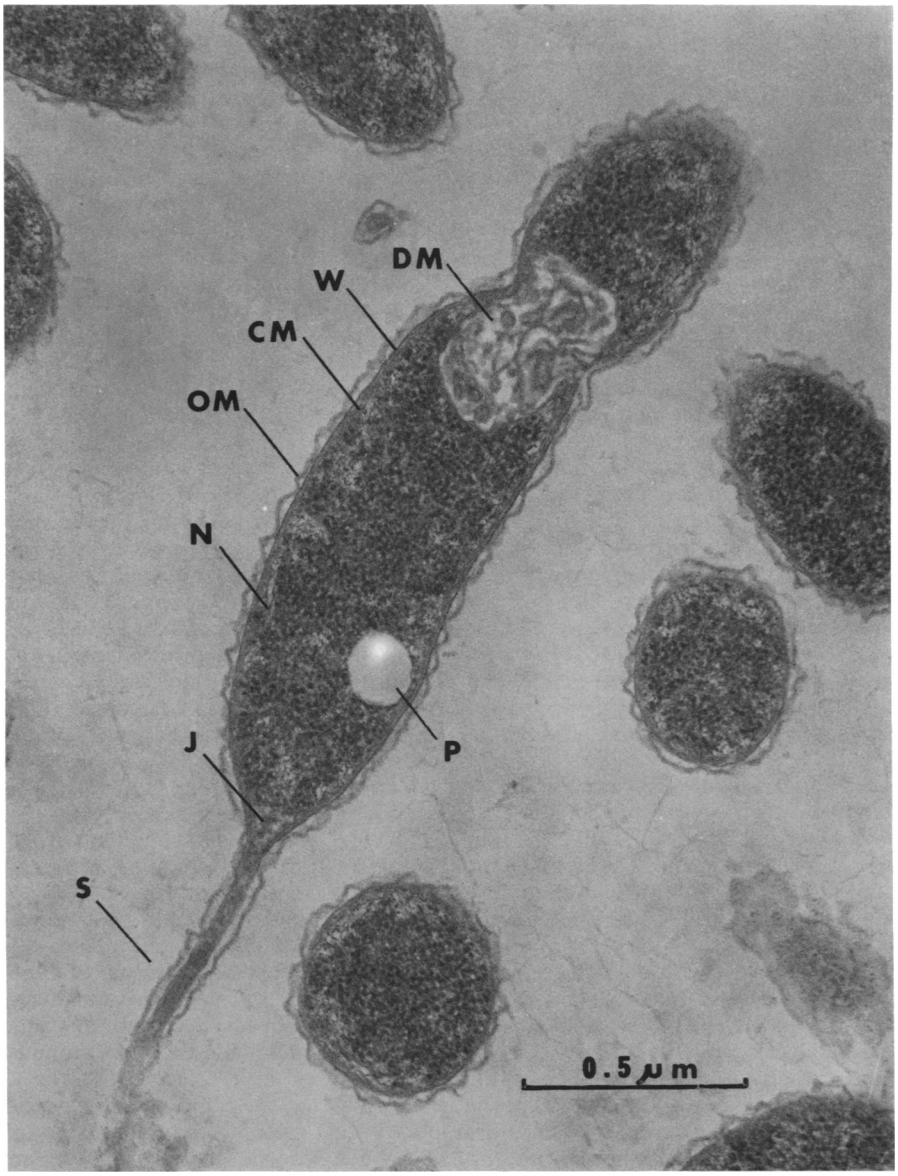


FIG. 3. *Longitudinal section through a dividing cell of C. crescentus. S, Stalk; J, ribosome-free region within the cell-stalk juncture; N, nucleoplasm; OM, outer membrane of the cell envelope; CM, cytoplasmic membrane; W, peptidoglycan layer of the cell wall; DM, division site mesosome; P, granule of poly-β-hydroxybutyric acid. The section was osmium fixed, stained with uranyl acetate, dehydrated with acetone, embedded in Vestopal W, and poststained with lead nitrate.*

than the equator of dividing cells and are especially numerous in cells growing under conditions of oxygen limitation (31) and in mitomycin C-induced filaments (83). Since both equatorial and random mesosomes are observable in whole cells as well as in sections, their presence cannot be ascribed to alterations during fixation and dehydration, as suggested for the smaller mesosomes of some gram-positive bacteria (see, e.g., reference 87).

## Composition of the Cell Envelope

**Lipopolysaccharide and phospholipids.** *C. crescentus* lipopolysaccharide was reported to contain mannose, galactose, glucose, D- and L-glycero-D-mannoheptose, and 2-keto-3-deoxy-

octulosonate as components of the polysaccharide moiety (D. Button and R. D. Bevill, Bacteriol. Proc., p. 57, 1970). Agabian and Unger (3) also found 2-keto-3-deoxyoctulosonate present in the cell envelope, but at only about 1/10 the amount relative to protein that is found in other gram-negative bacteria. The polysaccharide has not been further characterized.

Wood and Shapiro (287) mentioned that phosphatidylethanolamine, the major component of lipid A of *Salmonella typhimurium* (203), was not detected in *C. crescentus* lipopolysaccharide. In detailed reports of membrane lipid composition in *C. crescentus*, Contreras et al. (35) and Jones and Smith (118) substantiated this observation; phosphatidylglycerol was found to be the principal lipid, accounting for 70 to 86% of the membrane lipid in growing cells. Two other phospholipids accounted for almost all of the remainder: cardiolipin, and a derivative of phosphatidylglycerol identified as lysylphosphatidylglycerol (118); three minor components were also detected in variable amounts (35). The inner (cytoplasmic) and outer membranes were found to contain the same phospholipids, but in different proportions; the outer membrane contained a higher proportion of phosphatidylglycerol and its derivative and a lower proportion of cardiolipin (35).

**Proteins.** The most detailed description of cell envelope proteins so far available is the report of Agabian and Unger (3). Cell envelopes were prepared from two strains of *C. crescentus*, CB13B1a and CB15, by mechanical disruption in a French pressure cell according to the method of Koplow and Goldfine (128) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. In both strains, eight to nine proteins were found in the 74 to 130-kilodalton (kD) class; two other, smaller (45- and 39-kD), proteins were also present, as well as a 20-kD species in CB13B1a only (3, 35). Generally, the envelope proteins of *C. crescentus* strains are unusual in their large sizes; in other gram-negative bacteria, envelope proteins are almost all less than 60 kD (see, e.g., references 105, 204, 243, and 244). However, three (possibly five) of the envelope proteins of *C. crescentus* are penicillin-binding proteins (129), and, as in other gram-negative bacteria, penicillin-binding activity is localized predominantly in the inner membrane.

The aim of identifying the envelope proteins in caulobacters is to be able to determine whether and how the composition of the envelope changes during differentiation, particularly during stalk development. However, envelope protein composition in gram-negative bacteria is not constant; it has been shown to vary with strain (245), with mutations affecting other envelope components (128, 166, 280), with the presence of prophage (242), and with the composition of the medium (166, 245). For this reason, Agabian and Unger (3) examined the envelope proteins of *C. crescentus* cells grown in a variety of media. Because the composition of the medium influenced the profiles of both strain CB15 and strain CB13, they cautioned that such influences must be avoided in developmental studies that use manipulations of the nutrient environment to achieve synchronous populations.

**Peptidoglycan.** Peptidoglycan sacculi prepared from caulobacter cells by extraction with sodium dodecyl sulfate retain the shape of the cell, including the stalk and such features as septa (in *Asticcacaulis*) and constriction sites (in *Caulobacter*) and terminal swellings of the stalk in certain species (77; Fig. 4). The stalk bands (Fig. 1) do not copurify with sacculi, possibly because they are physically bound to the outer membrane of the stalk and are removed with it in the extraction procedure.

Hydrolysates of the peptidoglycan of *C. crescentus* contain three principal amino acids: alanine, glutamic acid, and *meso*-diaminopimelic acid in the molar ratio 2:1:1, respectively (3, 67). These results indicate that the peptidoglycan is of the A1$\gamma$, directly linked type, as was also found for *C. vibrioides* (125, 231). Essentially the same composition was found for peptidoglycan of *Hyphomicrobium* (120), except for the presence in these budding prosthecate bacteria of a significant amount of glycine.

Goodwin and Shedlarski (77) were unable to detect *meso*-diaminopimelic acid in peptidoglycan isolated from swarmer cells of *C. crescentus* CB2, although it was present in stalked cells of that strain. However, comparable amounts of *meso*-diaminopimelic acid were found in stalked and swarmer cells of *C. crescentus* CB13B1a (67). Whether the composition of peptidoglycan in the stalk itself is different from that of the cell body has not yet been examined by analysis of peptidoglycan prepared from isolated stalks.

**Summary.** *C. crescentus* exhibits several peculiar features with respect to cell envelope composition, although the composition of its peptidoglycan is not different from that of the majority of gram-negative bacteria tested. The lipopolysaccharide is composed principally of phosphatidylglycerol, rather than the more common phosphatidylethanolamine, and has a relatively low 2-keto-3-deoxyoctulosonate content. The majority of the envelope proteins are unusually large, greater than 70 kD. It is certainly too early to speculate on whether any of these differences are related to the peculiar capacity of the envelope for outgrowth in prosthecal development.

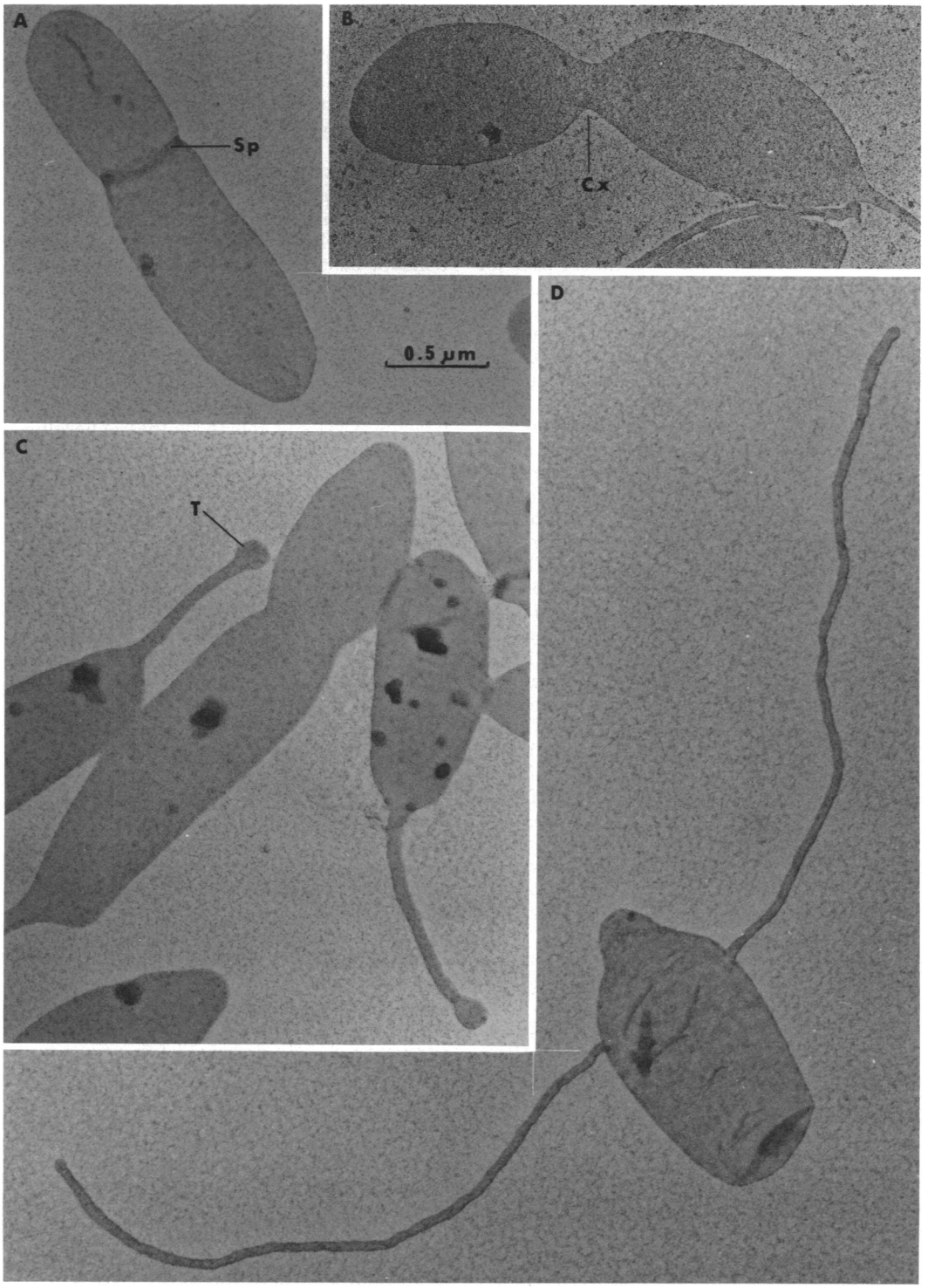


FIG. 4. *Peptidoglycan sacculi of caulobacters. (A) A. excentricus (Sp, septum). (B) C. crescentus (Cx, constriction). (C) C. bacteroides (T, terminal swelling of stalk, typical of intact stalks of this strain [CB11]). (D) A. biprosthecum. Sacculi were mixed with cytochrome c (0.5 mg/ml), mounted on nitrocellulose-coated Cu grids, and positively stained with uranyl acetate; when dry, they were rotary shadowed with Pt-Pd, 80:20.*

Most importantly, studies of this type have been limited to *C. crescentus*, and comparable studies with other caulobacters and other prosthecate bacteria are not yet available.

## Polar Organelles

The distinctive feature of caulobacter cells is the development of an envelope-derived prostheca. In all but one isolate (*A. biprosthecum*), this organelle arises from the cell pole. At the same pole, a single flagellum, holdfast material, pili (in many strains), and attachment sites for certain caulophages are present at the pole before prosthecal development. None of these five types of polar organelles is essential to viability, since each type can be removed by mechanical shearing or lost by mutation without a reduction in viability or growth rate.

**Flagellum.** The caulobacter flagellum is a transient cellular component, present and active only on the nonstalked pole of the dividing cell and during the period of motility that follows cell division. At the end of the motility period, it is typically shed into the culture medium. The released flagellum consists of a filament (19 nm diameter) and a hook (20 to 25 by 90 to 95 nm) at the cell-proximal end of the filament (142, 146, 224, 251, 253). The filaments typically contain two proteins: a major flagellin and a minor flagellin of slightly greater size. Lagenaur and Agabian (144) found two flagellins in each of 25 caulobacter strains; two strains (*Caulobacter bacteroides* CB11a and *C. leidyi* CB37) possessed only one flagellin. All caulobacter flagellins so far characterized lie in the range of 23 to 28 kD, at the lower end of the range for bacterial flagellins in general (14 to 56 kD [103, 257]). In the most extensively studied species, *C. crescentus*, the major flagellin is approximately 25 to 26 kD and the minor is 27 to 28 kD (71, 142, 145, 172, 209).

The two flagellins of most caulobacters are analogous to the "A" and "B" flagellins of *Bacillus pumilus* (reviewed in reference 257). As in *B. pumilus*, it is not clear whether both flagellins are present in each filament, or whether there are two types of filaments in a population. Structurally, there is a single type of filament, not a plain type and an elaborate type as in *Pseudomonas rhodos* (241). The problem cannot be resolved serologically, since the two proteins are highly cross-reactive (142). There are, however, three types of evidence that suggest that both proteins are present in a single filament. (i) In successive in vitro reconstitution of filaments from acid-solubilized flagellins of *C. crescentus* CB15, the proportion of flagellin A (26 kD) to flagellin B (28 kD) did not change significantly (ca. 24%) as the recovery of protein decreased 30-fold (253). (ii) The synthesis of the two flagellins began simultaneously in synchronous populations of *C. crescentus* CB13, but flagellin B (27 kD) synthesis was relatively brief, whereas flagellin A (25 kD) synthesis continued (145). Assembly of shear-sensitive external filaments closely followed synthesis, and the ratio of A to B increased as the filaments elongated; Lagenaur and Agabian (145) inferred that the presumably older, cell-proximal region of the completed filament contained both proteins, whereas the younger, more distal region contained flagellin A almost exclusively. (iii) Although some nonmotile mutants exhibit concomitant loss of both flagellins and restoration of both syntheses in spontaneous revertants (110, 142), at least one (PCM4) has been found unable to synthesize flagellin A yet still able to synthesize flagellin B (206). This implies not only that initiation of assembly may require the presence of both proteins, as suggested by Sheffery and Newton (253), but also that the two proteins are synthesized under the direction of two independent structural genes.

The amino acid composition of *C. crescentus* flagellins is similar to that of bacterial flagellins in general (257), in that seven amino acids (alanine, aspartate, glutamate, threonine, leucine, serine and glycine) account for nearly 80% of the residues (71, 142, 172, 254). Hook protein, which is antigenically distinct from the flagellins (146, 254), differs in composition mainly by the presence of significant amounts of proline and tyrosine (254); its size of 70 to 73 kD (111, 146, 254) is greater than those of hook proteins found in other bacteria (33 to 43 kD [51, 123, 241]). Nonmotile "polyhook" mutants possess the usual hook protein, but seem unable to terminate assembly at normal hook length (111, 254).

The envelope-associated region of the *C. crescentus* flagellum is a basal body consisting of five successive rings arranged along a central rod (111). The rod is occasionally seen on flagella released by swarmers (142, 146, 251), but the rings are not. The presence of five rings is unusual; most eubacterial flagella are associated with only four rings (49). It seems unlikely that any of the rings is identical with the concentric membrane ring structure seen in spirilla (39, 40), since the largest ring (32 nm) is less than one-half the diameter of a concentric membrane ring structure (70 to 80 nm).

When nonmotile mutants are selected in *C. crescentus*, four basic types are obtained either as unconditional or as thermosensitive mutants: flagellin negative (for flagellin A or both flagellin A and flagellin B); flagellin positive, flagellum

negative (assembly mutants); flagellin positive, stubby flagellum (filament elongation mutants); and flagellin positive, flagellum positive (nonmotile, but without discernible anatomic deficiency) (110, 136, 172, 251, 269). (All nonmotile mutants of *A. biprosthecum* are of the fourth type [214].) In each type, the majority of nonmotile strains are otherwise morphologically identical with the wild-type parent, and none of them appears to skip the I period (Fig. 2), the period of motility in wild-type strains.

**Prostheca.** In *Caulobacter* spp. and in *A. excentricus*, the prostheca originates at the same point on the cell pole which bears the flagellum (95, 96, 218). In *A. biprosthecum*, these sites do not seem coincidental. The active flagellum is subpolar, as in *A. excentricus*, but pseudostalks (one or two per cell [122, 152, 213]) are discernible only along the side of the cell, even while still very short. However, the position of the prostheca in this organism is not constant; the appendage migrates away from the adhesive pole toward (even beyond) the division site during growth (215). Accordingly, it is possible that even in *A. biprosthecum*, the sites of flagellation and of prosthecal initiation are coincidental.

Examination of the fine structure of the prostheca in caulobacters has revealed that it is constructed of envelope components: outer membrane, peptidoglycan layer, cytoplasmic membrane, and a core of internal membranes (31, 213, 223, 239). This appearance is different from the frankly cytoplasmic interior of prosthecae of *Prosthecobacter* (45), *Hyphomicrobium* and *Rhodomicrobium* (33), and *Prosthecomicrobium* and *Ancalomicrobium* (258).

Jordan et al. (122) found that the composition of *A. biprosthecum* appendages matches that of the cell envelope with respect to the proportions of dry weight accounted for by protein (45 to 49%), lipid (33 to 36%), and carbohydrate (14 to 19%). Their comparison of protein profiles by polyacrylamide gel electrophoresis revealed fewer proteins in prosthecae than in envelopes derived from whole cells; however, the protein profiles of *Caulobacter* species have not exhibited any significant differences between the stalk and the cell envelope (N. Agabian, personal communication; J. S. Poindexter and K. M. Lutta, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978 I34, p. 86).

Chow and Schmidt (29) found that the fatty acid composition of stalk fragments of *C. crescentus* was essentially the same as that of stalked cells. However, stalks and stalked cells contained a higher proportion of the cyclopropane fatty acid *cis*-9,10-methylenehexadecanoic acid and of octadecanoic acid than did swarmer cells and a correspondingly lower content of 14:0 and 16:1 acids. They suggested that some differentiation of lipids occurred in stalk development, particularly as the distal, biosynthetically inactive region of the stalk aged.

At present, the stalk appears to be an outgrowth of the envelope that can be distinguished by its shape and location, but not by its composition.

A peculiar feature of the caulobacter appendage not seen in prosthecae of other genera is the bands (Fig. 1). These structures are readily discerned in stalks of whole cells prepared for electron microscopy by shadowing or negative staining (see, e.g., references 95, 96, 119, 175, 213, and 218). In sections, they appear as annular structures or, more often, as continuous bands (31, 213, 223, 236, 239), presumably because the small diameter (10 to 20 nm) of the central pore precludes frequent achievement of a thin median section of a band. Nevertheless, it seems clear that the bands are typically perforate, and not complete septa, although they can serve as sites of core membrane attachment facilitating closure of the stalk after mechanical breakage (236). In this way, the bands clearly contrast with *Rhodomicrobium* prosthecal septa, which are thicker and interrupt the cytoplasmic membrane within the prostheca (19).

Jones and Schmidt (119) and Schmidt and Swafford (240) prepared isolated bands from stalks that had been sheared from cells. The bands were released from the isolated prosthecae by treatment with ethylenediaminetetraacetic acid, Triton X-100, and sonication. Isolated bands were then seen as disks composed of three to five concentric rings, essentially identical in diameter (ca. 80 nm) and appearance with the more recently described concentric membrane rings associated with the flagella of spirilla (39). Like concentric membrane rings, stalk bands are Triton X-100 insoluble, but unlike concentric membrane rings, the bands seem to be composed at least in part of lysozyme-soluble material (236, 240); both structures are associated with the outer membrane of the cell envelope.

Staley and Jordan (262) suggested that a band was formed each time a stalked cell divided. Swoboda and Dow (272), however, reported that they did not observe bands in the stalks of populations derived initially from a segregated swarmer cell population until after the third round of cell division. In swarmer populations of *C. crescentus* incubated under nutrient-limited conditions that allowed very little growth and no reproduction, the swarmers developed stalks, whereas none of them divided (Poindexter, unpublished data). Bands began to appear in the

elongating stalks by 4 h; by 24 h, the stalks were 12 to 18 μm in length and possessed an average of three bands each (Fig. 5). Thus, the insertion of the bands occurred at intervals during stalk elongation, even in nondividing cells. The addition of bands during prosthecal outgrowth may serve to prevent invasion of the stalk core by cytoplasm, thereby minimizing its capacity for catabolism (see below).

Using cell-free suspensions of prosthecae removed from *A. biprosthecum* in a Waring blender, Jordan et al. (122), Porter and Pate (226), and Larson and Pate (153) demonstrated several enzymatic activities of the prostheca. Of eight enzymes assayed and found present in cells, only four besides cytochromes (malate dehydrogenase [EC 1.1.1.37], succinate dehydrogenase [EC 1.3.99.1], alkaline phosphatase [EC 3.1.3.1], and reduced NAD dehydrogenase [EC 1.6.99.3]) were also detectable in prosthecae (122). Isocitrate dehydrogenase (EC 1.1.1.41), glucose-6-phosphate dehydrogenase, lactate dehydrogenase (EC 1.1.1.27), and reduced NAD oxidase were present in the cells, but not the prosthecae, implying that the catabolic systems of the prosthecae were incomplete. This was consistent with the later observation of Porter and Pate that exogenous glucose accumulated by prosthecae was not metabolized (226).

The glucose uptake system was characterized in considerable detail with respect to specificity, kinetics, energy dependence, and sensitivity to inhibitors (153). Two systems were distinguished: a high-affinity system ($K_m$ = 1.8 μM) and a low-affinity system ($K_m$ = 34 μM). Supported by energy from respiration, they were able to concentrate glucose to more than 200 times the ambient concentration. The prosthecal uptake systems lacked a substrate specificity comparable to those of permease systems found in membrane vesicle preparations from such bacteria as *E. coli*; glucose accumulation by both the high- and low-affinity systems was competitively inhibited by galactose and by xylose, although not by fructose.

On the basis of these studies, it was concluded (153, 226) that the prostheca functions as an extension of the cell's absorptive surface and that within the prostheca the absorbed substrates are not dissimilated. In the dilute natural environment, the prostheca would establish a localized concentration gradient allowing diffusion of nutrients toward the catabolically active cytoplasm of the prosthecate cells.

**Holdfast.** In thin sections, the holdfast site at the distal end of a *Caulobacter* stalk is characterized by a smooth contour of the outer membrane and the presence of a relatively electron-opaque material in the space between the outer membrane and the peptidoglycan (223). In *A. excentricus* (223) and *A. biprosthecum* (279), a similar site can be identified on the cell pole associated with darkly staining amorphous or granular material external to the site (223, 279). The composition of holdfast material remains uncertain, although its staining properties indicate that it is an acidic polysaccharide (279); such substances commonly serve as adhesive materials in bacteria (38).

Spontaneous and nitrosoguanidine-induced mutations in adhesiveness were found in *A. biprosthecum* and *C. crescentus* CB15 by Umbreit and Pate (279). Nonadhesive cells were enriched by subculturing the supernatant fluid of cultures containing cheesecloth, to which the wild-type cells adhered. The mutants thus obtained were unable to initiate rosette formation, although they could enter rosettes initiated by wild-type cells. Thus, the $Hol^-$ mutants retained localized, but passive, adhesiveness.

**Pili.** Caulobacter pili arise at the same location on the cell surface as the holdfast material (232, 279). They are most readily found during the motility period at the base of the flagellum, and only infrequently on stalked cells of *Caulobacter*, at the distal end of the stalk (232).

The pili of *C. crescentus* are composed of a single 8.5-kD protein (pilin) that contains some carbohydrate, but no detectable phosphorus (143). The amino acid composition of pilin is similar to that of the flagellins (147). In contrast to the transient presence of pili, Lagenuar and Agabian (143) found that pilin was present intracellularly at all times and that its synthesis occurred continuously through the cell cycle. The pili of *A. biprosthecum* are more persistent than those of *C. crescentus* during growth (279), but in stationary phase they are released into the culture medium (214).

Pili (fimbriae) in bacteria are generally involved in attachment of piliated cells to particles (22, 30, 55, 56). This seems to be the case also for caulobacter pili, which arise from the holdfast site, are found extending from cell to cell in rosettes (279), and form complexes with RNA phages and some DNA phages. The apparently ancillary roles of pili in both rosette formation and phage adsorption suggest that they assist in initial stages of attachment; lasting adhesion of the cell is mediated by the more persistent holdfast material, and irreversible adsorption of phages probably occurs at receptor sites on the cell surface.

**Caulophage receptors.** Attachment of a few caulophages to susceptible cells has been reported to occur at random sites on the cell surface (112, 282), but attachment of many of the phages occurs predominantly or exclusively

at specific sites. Three such sites have been reported: the holdfast area of the nonstalked pole, pili, and the flagellum. The phages that have been identified as attaching specifically or preferentially to the holdfast site of motile cells are φCbK (5; reviewed in reference 248), 30 of

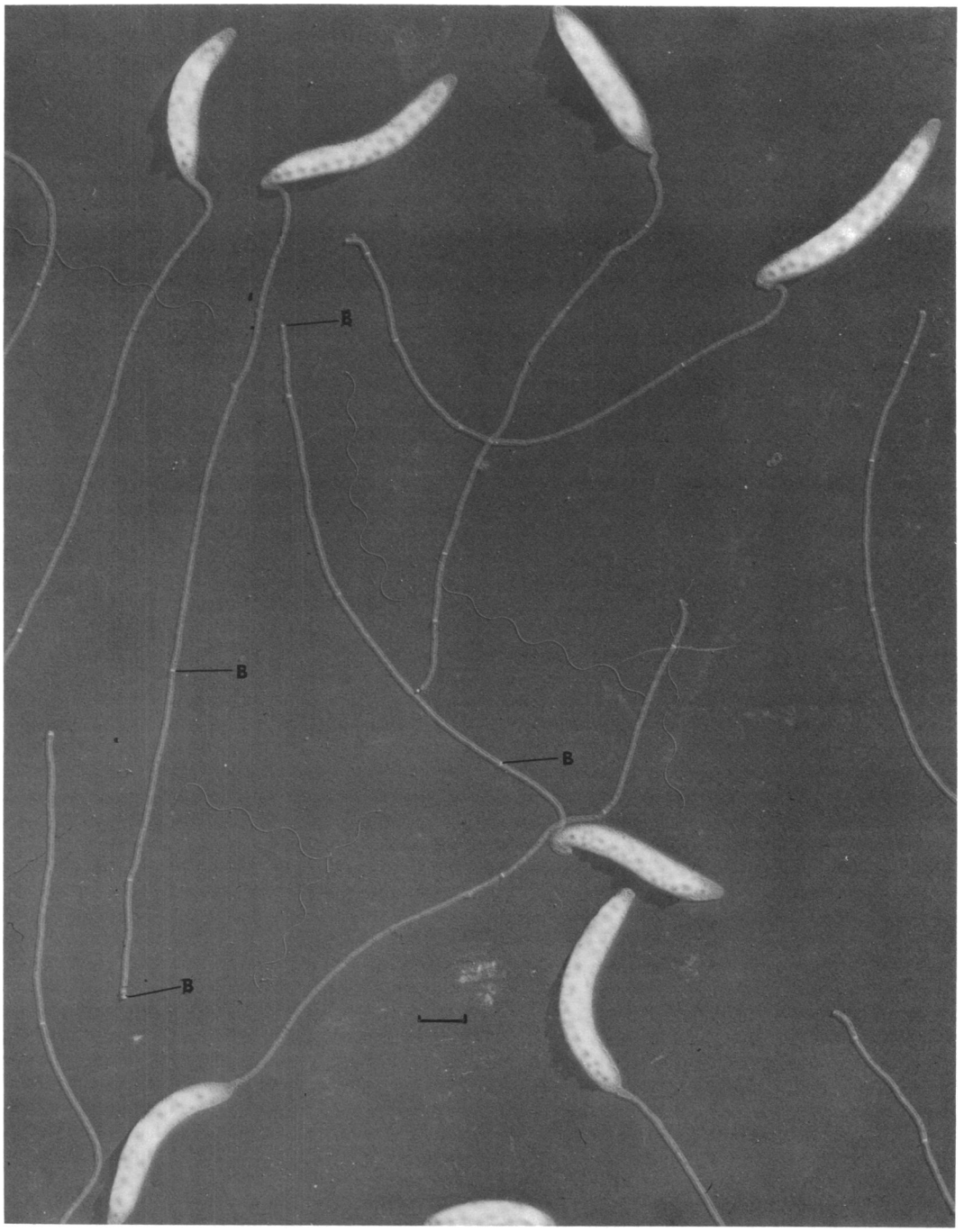


FIG. 5. *Stalk bands (B) in C. crescentus. Swarmer cells were harvested from early stationary phase in Hi-glucose (0.3%)-glutamate (0.3%) medium and reincubated in cell-free medium from the same culture. Viable counts at six points during the 24 h of incubation remained constant at 2.4 × $10^9$ colony-forming units per ml, and motile cells were not produced. Marker is 1 μm.*

the 36 DNA phages isolated by Johnson et al. (112), and each of the six group II *Asticcacaulis* phages examined (279). All of the RNA phages that have been examined (98, 112, 172, 232) and several DNA phages (147, 178, 214) have been seen attached to pili. Four DNA phages (72, 117, 214) can attach to flagella, but attachment to free flagella appears to be reversible and neither activates nor inactivates the phages. Empty capsids of the flagellotropic phages are seen only when virions attach to the cell pole; in *A. biprosthecum*, such attachment can occur at either cell pole (see Fig. 5 of reference 214). This list of site-specific phages includes all morphological type I phages (Table 2) that have been tested.

In considering the evidence that caulophages attach preferentially to the flagellated pole, it is important to note that this is the site of greatest adhesive activity in caulobacters. Also, as discussed above (see also reference 131), attachment of cells of a caulobacter population to any kind of substratum or particle is accounted for predominantly by actively motile swarmers. Not only does the swarmer possess both types of attachment organelles present in these bacteria, but also the volume of medium occupied by a motile cell during a finite period of time is greater than that occupied by a nonmotile cell; the probability of its colliding with another particle is increased proportionately. To eliminate the difference in collision frequency between motile and nonmotile populations, the ratio of virions to cells in adsorption mixtures with segregated swarmer and stalked cell populations should, ideally, be adjusted to equalize the probability of collision in the two mixtures. To date, determinations of adsorption rates of caulophages in segregated populations have regularly and conscientiously used the same phage and cell concentrations for both populations. With few exceptions (5, 8, 98, 147, 214), adsorption rates were higher in populations of motile cells than in populations of nonmotile cells, whether nonmotility resulted from stalk development (113, 147, 247), removal of flagella by mechanical shearing (72, 117, 214, 232), increased viscosity of the medium (72), starvation (249), chemical inhibition (75, 117, 139, 147), or incubation at a temperature that reduced motility (73, 75, 147). It has not been reported whether the phages seen to attach to random sites on caulobacter cells appear to attach preferentially to motile cells.

The fundamental question in this matter is whether attachment of phages to holdfast sites, pili, or flagella is necessary for phage infection or whether it promotes infection by concentrating phage particles near the cell surface, increasing the probability that at least one virion will reach an effective receptor (entry) site. The latter possibility has been the conclusion reached with respect to the flagellotropic phages (72, 117, 214) and also the tentative inference with respect to phages attaching to pili (16, 147, 214, 232). The isolation of mutants that lack flagella but are susceptible to flagellotropic phages (72, 110), of mutants that lack pili but are susceptible to pilus-attaching phages (147), and of nonadhesive mutants susceptible to holdfast-attaching phages (214, 279) offers clear evidence that the receptor sites are distinct from phage-attaching sites, such as flagella, pili, and holdfast material. The receptor sites appear to constitute a fourth functional class of structural components of the nonstalked pole; proof of their distinctiveness from the other organelles awaits their purification and studies of their chemical and serological properties.

**Pleiotropic mutations affecting polar organelles.** It is clear that there is some degree of structural or genetic interdependence among all five types of polar structures in caulobacters. The majority of nonnutritional mutants isolated exhibit pleiotropic changes, whether selected according to their phage susceptibility, motility, buoyancy, or adhesiveness, and both with and without mutagenesis. Examples of pleiotropic mutations are listed in Table 7. Additional instances of pleiotropy are described by other authors (5, 69, 72, 74, 98, 101, 109, 112, 117, 136, 139, 143, 172, 178, 221, 233, 234, 248, 251); pleiotropy sometimes includes physiological as well as morphological traits and phage susceptibility (see, e.g., references 101, 111, 112, 142, 233, 234, and 254).

Intentional selection for pleiotropic mutants, as nonmotile survivors of phage infection, can result in isolation of some mutants with still further phenotypic changes. Five of the 30 isolates obtained by Fukuda et al. (70) by such a procedure were found unable to develop stalks in complex medium, able to assemble but not to activate flagella, and to lack adhesiveness. Their properties imply either that there is a process or a structure common to motility and phage infection whose impairment interferes with differentiation of the envelope or that several mutations had occurred in these mutants. Mutant frequencies were not reported. A similar double-selection procedure resulted in isolation of a mutant possessing exceptionally large numbers of pili, increased susceptibility to phage $\phi$Cb5, and defective stalk formation in PYE medium (143).

Spontaneous motile revertants from pleiotropic nonmotile mutants usually exhibit full restoration of parental properties, but many re-

TABLE 7. *Examples of pleiotropy among caulobacter mutants*

| Selection | Parent | Mutagen | No. of mutants showing[a] unselected alteration as loss or reduction of: | | | | No. of mutants pleiotropic/no. of mutants studied | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | Motility | Piliation | Adhesiveness | Phage susceptibility | | |
| Survival of phage infection | *C. crescentus* CB13B1a | NTG[b] | 33 | NR | NR | 100 | 33[c]/107 | 73 |
| | *C. crescentus* CB15 | None | ~50 | NR | NR | 149 | 50[c]/150[d] | 62, 63 |
| | *C. crescentus* CB15 | NTG | 22 | NR | NR | 50 | 22[c]/50 | 62, 63 |
| | *C. crescentus* CB13B1A and CB15 | None | 12 | 196 | 0 | 196 | 12[c]/196 | 147 |
| | *A. biprosthecum* | None | 0 | 2 | 2 | 2 | 2[c]/2 | 279 |
| Nonmotility | *C. crescentus* CB15 | None | X | NR | 0 | 62 | 62/69 | 110 |
| | *A. biprosthecum* | None | X | 0 | 0 | 0 | 0/83[e] | 214, 279 |
| Inability to initiate rosette formation | *C. crescentus* CB18 | None | 5 | 5 | X | 5 | 5/5[f] | 279[g] |
| | *A. biprosthecum* | None | 0 | 3 | X | 10 | 10/14 | 279 |
| | *A. biprosthecum* | NTG | 0 | 0 | X | 0 | 0/2 | 279 |

[a] NR, Not reported, X, selected alteration.
[b] NTG, *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine.
[c] Number exhibiting alterations other than phage susceptibility.
[d] Of the 150 isolates, 1 also exhibited aberrant cell division.
[e] All of these mutants assembled inactive flagella.
[f] Two of the five isolates also exhibited aberrant stalk development and cell division.
[g] Also J. L. Pate, personal communication.

cover only motility, or motility and only partial phage susceptibilities (62, 63, 69, 73, 136). Failure of spontaneous revertants in a specific trait to be otherwise identical to the parental strain is seen in auxotrophic as well as morphological mutants (101). Clearly, some pleiotropy is due to multiple mutations, and some reversion is mutation in other than the mutated genes, possibly in suppressor-like loci.

Multiple phenotypic changes in organelles at the cell pole could result from any one of several mechanisms (147); five suggest themselves from observations already accumulated. (i) The gene governs a membrane function, e.g., energy transformation or supply or transport of relatively large molecules, such as protein subunits, required for development of polar organelles. (ii) The gene governs the formation of a structure within the pole upon, through, or by which the affected organelles are assembled. (iii) The gene governs the synthesis or assembly of a polar organelle whose presence at the pole is required for the formation of another polar organelle(s). (iv) The gene governs the primary structure of more than one polar organelle, and differences in their eventual compositions normally result from post-translational processing. (v) The gene governs transcription of more than one of the structural genes for polar organelles, providing coordinate regulation of their expression.

Any of the first four mechanisms would contribute to phenotypic coordination of developmental events at the cell pole; spontaneous revertants in the single affected gene could exhibit complete restoration of parental characteristics. Recognizing mutations in a coordinating gene among the pleiotropic mutants will require more elaborate genetic analysis than characterization of spontaneous revertants (e.g., gene isolation in clones; see next section).

Altogether, the pleiotropic mutants provide the best resource for determining whether the coordinated expression of the events of polar morphogenesis in caulobacters is controlled at the transcriptional level by gene interactions or whether sufficient coordination is provided by coincidence of location of the structures and common or interdependent structural requirements for their development.

## Deoxyribonucleic Acid and Nucleoid Structure

In the DNA of *C. crescentus* (62 mol% guanine plus cytosine [218]), $N^6$-methyladenine accounts for approximately 1% of the adenine residues and 2% of the cytosine is 5-methylcytosine in both stalked and swarmer cells (46). All of the DNA is chromosomal; resident extrachromosomal elements have not been detected (57, 197, 286). Wood et al. (286) reported that *C. crescen-*

*tus* DNA contained hairpin loops, interpreted as inverted repeat (IR) sequences, of 100 to 600 nucleotide pairs. The number (ca. 350) of such loops per genome could account for the 4% of the DNA that renatured rapidly with unimolecular kinetics. The proportion of sheared, denatured DNA that bound to hydroxyapatite columns did not vary with fragment length, implying that the inverted sequences were not randomly distributed along the genome. Reassociation kinetics were identical for stalked cell and swarmer DNAs.

These latter two observations were confirmed recently by Nisen et al. (197), who succeeded in cloning two fragments (9.5 and 4.7 kilobases) containing the inverted repeat sequences in a λ-WES vector. The two segments hybridized to the same four regions of the chromosome, further evidence that the inverted repeat regions are clustered. These four regions appeared identical in DNAs from stalked (including predivisional) cell DNA and swarmer DNA. However, the longer of the cloned segments (λ-WES·IR-1) also hybridized to two additional regions of swarmer cell DNA, indicating that at least part of the sequence present in IR-1 is translocated during the swarmer stage (197). Whether this apparent translocation was preceded by duplication or whether only a fraction of IR-1 was translocated could not be determined.

In studies of envelopes prepared without disruption of their association with the DNA, Evinger and Agabian (60, 61) found a marked difference in the sedimentation behaviors of the "envelope-associated nucleoids" (EANs) of swarmer and stalked cells. The EANs prepared from stalked cells exhibited a sedimentation coefficient of ca. 3,000S, whereas those from swarmer cells exhibited a sedimentation coefficient greater than 6,000S. This difference in sedimentation behavior was eliminated by treatment with ribonuclease A and deoxyribonuclease I (EC 3.1.4.5); both types of nucleic acid-free envelopes sedimented at ca. 1,975S. The DNA of stalked cell EANs spread out from the envelope in a cytochrome film as smooth loops and apparently flexible strands, whereas the DNA of swarmer EANs appeared rigid and somewhat tangled (61).

Since only 4% of the DNA is accounted for by inverted repeat sequences, it seems unlikely that the presence of excess inverted repeat regions in swarmer DNA could account for the difference in physical organization of swarmer and stalked cell EANs. Examination of the proteins in EANs (61) prepared from continuously labeled cells revealed the presence of several nonenvelope proteins, including the DNA-dependent RNA polymerase (RNA nucleotidyltransferase [EC 2.7.7.6]). The only clear difference in the profiles was the presence of a 26-kD protein (possibly flagellin A) in the swarmer envelopes; this would not explain the difference in sedimentation behavior of the two types of EANs. The role of the RNA of the nucleoids has not yet been explored. It is conceivable that the higher ratio of RNA to DNA in swarmer (ca. 1:2) than in stalked cell (ca. 1:4) EANs (60) reflects intranucleoid organization in the swarmer that results in greater restriction on the unfolding of the DNA.

## Quantitative Distribution of Cell Components to Stalked Cell and Swarmer Siblings

An explanation for the difference in developmental and reproductive behavior of swarmer and stalked cell products of fission has been sought through determination of whether cell components (as membranes, DNA, or cell volume) were shared in equal amounts by the respective progeny.

**Membranes.** Galdiero (76) prepared synchronous swarmer populations from exponentially growing cultures of *C. crescentus* CB13 labeled with [$^{3}$H]leucine or [$^{32}$P]phosphate. Cell envelopes were prepared at intervals during one synchronous reproductive cycle and from segregated populations of the stalked cell and swarmer progeny of that cycle. During the cycle, the specific radioactivity of the envelope proteins ($^{3}$H) decreased 50% and was identical in stalked and swarmer progeny. The specific radioactivity of envelope lipids ($^{32}$P) decreased in parallel with that of the proteins, but was slightly lower in stalked than in swarmer progeny. Nevertheless, there was no evidence of significantly different quantitative distribution of parental envelope components to either type of progeny cell. The same observation with respect to the phospholipids has been reported for *C. crescentus* CB13 and CB15 (171).

As shown also by the constant, approximately equatorial position of the division plane in the studies of Terrana and Newton (276), Galdiero's measurements demonstrated that the distinction between the progeny as mother and daughter cells used by some authors (53, 175, 272, 283) is inappropriate (90). The difference in site of growth in fission bacteria and budding bacteria was clearly demonstrated in the studies of Moore and Brubaker (183). Exposure of cells to *cis*-platinum(II) diamminodichloride resulted in the growth of *C. crescentus* (CB2) as multinucleate, unconstricted filaments, whereas this type of growth occurred in *Hyphomicrobium* sp. (B-522) only in the buds, not in the mother cells.

Removal of the inhibitor was followed by irregular fragmentation of the *Caulobacter* filaments, eventually yielding cells of normal length, but resumption of reproduction by *Hyphomicrobium* cells required development of new hyphae and subsequent production of normal-sized buds. Thus, growth and reproductive potential of a *Caulobacter* cell are distributed along its entire length; in *Hyphomicrobium*, growth occurs in the bud, whereas reproductive capacity resides in the mother cell.

**Deoxyribonucleic acid.** Light microscopic studies revealed that the number of stainable nucleoid bodies is the same in swarmers and in nondividing stalked cells (97, 99). Similarly, the number of gamma-ray targets was found to be equal to one per nondividing cell (100).

Osley and Newton (205) examined the question of DNA distribution in two ways. First, they determined the radioactivity in three generations of swarmers released by stalked cells in which 10% of the genome was labeled. Each succeeding generation exhibited a radioactivity accumulation curve with one-half the slope of the previous generation, a pattern consistent with random segregation of the originally labeled strand of DNA. This experiment was repeated by Iba et al. (99) with essentially identical results.

In the second method used by Osley and Newton (205) and later by Iba and Okada (102), swarmer cells were prepared with one completely labeled strand of DNA. The swarmers were incubated in unlabeled medium, and samples of the initial swarmer population and of the mixed population resulting from their reproduction were examined by autoradiography. Distribution of radioactivity within the populations implied that the genome segregated as a unit and that neither strand of DNA was preferentially inherited by the stalked or swarmer offspring cell. However, the number of grains detected per radioactive cell remained constant in one study (205), but decreased by 50% in the second study (102); thus, the number of chromosomes per cell is not yet certain.

**Cell volume.** Terrana and Newton (276) reported that when a *C. crescentus* cell divides, the incipient separation site is asymmetrically located in such a position that the stalked progeny will be 1.13 times as long as the swarmer progeny. Since the diameters of the respective progeny are equal, the stalked sibling would be 13% larger than the swarmer. They suggested that such a difference in size would partly explain the observed difference in cycle times (131, 218), since the stalked progeny would have less growth to accomplish than the swarmer. However, if growth were continuous and growth rates were equal, the 13% difference in size would result in a stalked cell cycle time that was 89% of the swarmer cycle time. The observed proportions are 75% in complex medium and 67% in glucose minimal medium (see Table 8). Thus, the size difference observed by Terrana and Newton could account for very little of the difference in cycle times.

A consistent difference in size between stalked and swarmer siblings of growing populations of wild-type *Caulobacter* had not previously been reported. To reexamine this question, I measured the respective lengths of incipient or just-divided siblings in the illustrations of 11 other reports of developmental studies on *Caulobacter* that included electron micrographs (81, 131, 136, 138, 139, 172, 175, 206, 218, 247, 248). Of 36 measurable cells, the stalked cell/swarmer sibling length ratio of 11 cells was equal to 1.00, that of 12 cells was greater than 1.00, and that of 13 cells was less than 1.00. The mean ratio for all 36 cells was 1.00.

Terrana and Newton detected unequal progeny size in both exponential and synchronous populations. In the course of experiments on the effect of nutrient shifts on morphology (222), it has been observed in this laboratory that unequal progeny size is not uncommon in populations experiencing a change in nutrient supply. However, among 43 dividing cells of a control population, the ratio of stalked cell to swarmer sibling length was greater than 1.00 in 25 cells (mean = 1.08), equal to 1.00 in 6 cells, and less than 1.00 in 12 cells (mean = 0.90); the mean ratio in the population was 1.02 (Poindexter, unpublished data). It is possible that the populations examined by Terrana and Newton were experiencing a physiological transition not intended by the investigators. In general, fission in *C. crescentus* under conditions allowing unrestricted subsequent growth results in progeny of the same size.

In contrast to *C. crescentus*, cell division in *Asticcacaulis* is typically unequal, with the swarmer being considerably smaller than the stalked sibling. In *A. biprosthecum*, the stalked sibling is 1.82 times longer than the swarmer (213, 215); the swarmer cycle time is not known for this isolate. In *A. excentricus*, the stalked sibling is 1.50 times longer than the swarmer (218). This difference would require that the ratio of cycle times be 0.66 (stalked/swarmer), not the much lower 0.49 observed (218). Again, as in *Caulobacter*, the swarmer takes longer to complete a division cycle than would be predicted if growth were continuous and growth rates were equal.

### Conclusions Regarding Relationships Between Structure and Composition and Cellular Differentiation

Of the several features of structure and composition of caulobacters that have been examined to date, the most significant difference between stalked cells and swarmers appears to be the physical organization of the nucleic acids associated with the cell envelope. The apparent relative compactness of the swarmer nucleoid may be due to the small differences in the proteins present or to the presence of a higher proportion of RNA; this remains to be determined. Nevertheless, this observation has extensive implications for the differences in behavior of the cell types released from each other by cell fission. For example, the difference in physical organization of the nucleoid may determine the relative availability of some or all of the DNA not only for replication but also for transcription, even though extracts of the two types of cells have quantitatively equivalent RNA polymerase activities (17).

If the condition of the nucleoid does influence subsequent biosynthetic events, it then becomes important to determine, as well, how the dividing cell can discriminate between the two nucleoids and arrange them in different physical associations with RNA and with the cell envelope. It seems likely that the cytoplasmic membrane is an important participant in guiding and arranging the bacterial nucleoid (34, 52, 79), and the presence of elaborate internal membranes in the caulobacters may be of greater significance than has yet been recognized.

## EXPERIMENTAL STUDIES OF CELLULAR DIFFERENTIATION

Stalk development in caulobacters appears to reflect two levels of control such that, whereas the initiation of development occurs in practically every cell regardless of environmental signals, stalk elongation constitutes a morphogenetic response to nutrient decline. The apparently obligatory initiation of development must reflect some degree of strictly internal regulation of morphogenesis, and it is particularly this aspect of cellular differentiation that has been investigated (reviewed in reference 246), almost exclusively in *C. crescentus*.

Because an exponentially growing population of caulobacters is a mixture of stalked cells and swarmers, such populations are not suitable for studies of the course of development. Two methods for mathematical prediction of morphological stages within exponentially reproducing populations have been formulated (207, appendix; 150). The different methods predict that 25 to 35% of an exponential population would be in the swarmer stage, 38 to 60% would be growing stalked cells, and 15 to 32% would be in the process of cell division. It is not clear whether such wide ranges are due to differences in the mathematical approaches, to the experimental data used in the calculations, or to heterogeneity of developmental kinetics within populations in exponential phase. Both proposals were based on the assumption that the medium remains constant and unaltered by an expanding population so that each successive doubling occurs in the same environment; this would be a safe assumption only if steady-state conditions were ensured by experimental design.

The problem of population heterogeneity can, however, be circumvented. As demonstrated by Stove and Stanier (269), mechanical separation of the swarmer cells from an exponential culture yields a population that is homogeneous with respect to developmental stage and to age (as time interval before cell division). Subsequently, a large proportion of developmental studies with caulobacters have used swarmer populations, and a variety of methods for obtaining such populations have been devised. In contrast to studies of development in swarmer populations, very limited experimental attention has been given to the course of events in the stalked siblings. It has been assumed, either implicitly or explicitly (27, 60), that a stalked cell resulting from development of a swarmer is not different from a stalked cell arising by cell division. There has not been a firm experimental basis for this assumption, and recent evidence regarding phospholipid synthesis (171) suggests it should be discarded. Nevertheless, the advantage of the assumption has been to focus the work of several laboratories on the swarmer-initiated cycle; these are the studies that will be reviewed here.

### Establishment of Synchronous Populations

**Methods.** Two basic methods have been used for the preparation of swarmer populations: differential or gradient centrifugation, and collection of progeny released by cells attached to a substrate. These methods are similar in practice to those used for other types of microorganisms (see, e.g., reference 84). Centrifugation of caulobacters results in discontinuous distribution of cells, with the swarmers sedimenting faster than the stalked cells. The difference in sedimentation rates is probably due to the effect of cell shape on frictional coefficient, since cells whose stalks have been removed by shearing (Poindexter, unpublished data) or have been shed as a consequence of mutation (221) sediment as rapidly as swarmers.

Centrifugation methods are suitable for reso-

lution of more than 90% of the cells of a heterogeneous population of any volume or density into segregated populations of swarmer and stalked cells, either by differential centrifugation (5, 41, 46, 76, 218, 232, 247, 251, 269, 282) or by centrifugation through Ludox gradients (60; see also references 1, 61, 143, 145, and 146). Both procedures involve a period during which cellular metabolism is reduced by chilling, but this has only a minimal effect on subsequent synchronous behavior of the swarmer population (218; see Table 8).

A population of attached caulobacters washed repeatedly or continuously with medium consists almost entirely of stalked cells, whereas the progeny released by an attached population consists of swarmers. Even if some of the swarmers attach to the substrate, no detectable detachment of stalked cells occurs, and the harvested liquid phase comprises a homogeneous population of swarmers whose age distribution can be selected experimentally by the frequency of replacement of the liquid. This sort of procedure yields segregated swarmer cell populations only.

In the swarmer elution technique of Degnen and Newton (48), cells growing attached to large petri plates are washed with fresh medium at constant temperature. Swarmers are harvested by collecting the medium from the plate after a 10-min (27, 193, 205, 207, 209) or 7-min (99, 100, 101) incubation period. Accordingly, the swarmers harvested should all be within 7 to 10 min of age, i.e., within 7 to 10 min since completion of cell division. Since the temperature is constant and the medium is not exchanged between harvesting and incubation of the swarmer population, the procedure does not involve any shift that should affect the physiological state of the cells. Swarmer elution techniques have been developed with other substrates, e.g., cheesecloth (147), glass beads (262), filters (272), and the walls of a narrow culture vessel (98).

The degree of synchrony and the reproducibility of the methods used are reflected in the summary in Table 8, below. The data presented are taken from reports that provided either particle or viable counts determined at least into the reproductive plateau after the single synchronous wave of the cell division that occurs in swarmer populations of *C. crescentus*. The differences between the behaviors of *C. crescentus* CB13 and *C. crescentus* CB15 populations are probably attributable to the use of different media with the two strains.

**Indicators of synchrony.** The two principal measurable parameters of the time course of reproduction in synchronous populations are the time of onset of cell division and the duration of the round of division (18). During the interval before the onset of division, the entire series of events comprising the development and maturation of caulobacter swarmers occurs. These events comprise, in temporal order: (i) motility accompanied by some cellular growth (76, 101, 218, 247, 276); (ii) loss of motility (100, 145, 193, 218), approximately simultaneous with the initiation of stalk development and DNA synthesis (48, 193, 214, 247, 262, 269), accompanied by biosyntheses at maximal rates (48); and then (iii) subsidence of DNA synthesis and reduction in overall biosyntheses (48, 76, 101) as flagellar activity resumes and cell surface constriction proceeds toward completion (100, 145, 193, 218). The resulting progeny population is heterogeneous, consisting of swarmer and stalked cell siblings. The time course of these events in Hp-glucose-grown *C. crescentus* CB15 swarmer populations is presented in Fig. 6 and 7, below.

These three general subsets of events coincide with the three periods of the general eubacterial cell cycle recognized by Helmstetter et al. (84) and designated by them the I, C, and D periods, respectively. Those designations were devised to indicate the period of accumulation of a hypothetical initiator of DNA synthesis (I), the period of chromosome replication (C), and the period of events associated with cell division (D). These seem altogether appropriate to the *C. crescentus* swarmer cycle; however, several of the discussions (see, e.g., references 48 and 99) of the *C. crescentus* cycle have borrowed the designations G1 (first growth), S (DNA synthesis), and G2 (second growth) from the labels for stages of the eucaryotic cell cycle. These are inappropriate, since most of growth occurs during DNA synthesis in *C. crescentus*, as in other bacteria, and there is no equivalent in time of the eucaryotic M (mitosis) period. Accordingly, the designations generally used for the bacterial cycle (I, C, and D) will be used here.

The duration of the initial round of cell division in *C. crescentus* swarmer populations appears to be more reproducible in glucose minimal medium than in PYE, but does not seem dependent on the method used for preparing the population. Most significantly, synchrony is not improved by using those swarmers released from their siblings within 7 to 10 min of each other rather than by collecting all the nonstalked cells by centrifugation. On the average, the duration of the doubling period is more than 50 min in Hp-glucose when swarmers are harvested for only a 10-min interval. The ratio of the two intervals (greater than 5) reflects a decay of the expected synchrony that presumably results from individual variation among the swarmers. One consequence of this decay is that DNA synthesis often resumes in the population before

the cell number has doubled (see Table 8). This overlap is interpreted (48, 99, 193) as evidence that the stalked cell cycle differs from the swarmer cycle by consisting only of C and D periods, lacking the preparative I period.

## Events in Synchronous Swarmer Populations

In addition to cell division, several other events have been analyzed in synchronous swarmer populations, and the majority have been found to exhibit some degree of periodicity. Among the clearly periodic events are DNA synthesis from exogenous precursors, change in nucleoid organization, rate of phage adsorption, and synthesis and assembly of the components of the flagellar apparatus and of certain proteins whose functions are not yet known. These are summarized in Fig. 6 and 7 and Tables 8 and 9. Stalk development is also regularly observed to begin in practically all the cells during a relatively brief period within the cycle (48, 193, 218, 247, 262, 269), and motility is lost suddenly by the population, to resume by the time of onset of cell division (100, 145, 193, 218, 269).

**Deoxyribonucleic acid synthesis.** DNA synthesis is negligible during the period of motility and begins as motility reaches a minimum and stalk development becomes detectable (49, 193). A discrete onset of DNA synthesis is detectable in these populations regardless of the strain, the medium, or the method for obtaining swarmers (60, 99, 209; Table 8). There is also a period, roughly during the time of cell division, when DNA synthesis decreases sharply (48, 60, 99, 193, 209). The apparent duration of DNA synthesis is influenced by the precursor provided, as well as by whether synthesis is monitored by continuous labeling or by pulse-labeling (48, 193, 209), as illustrated in Fig. 7. Nevertheless, it is clear that uptake of exogenous nucleosides or adenine and incorporation into acid-insoluble, base-stable material is periodic.

It is possible that delay in uptake of certain precursors reflects inducible uptake systems. Degnen and Newton (47, 48) had reported that *C. crescentus* CB15 cells grown in Hp-glucose did not incorporate exogenous thymine, thymidine, or deoxyribose into DNA. However, Haars and Schmidt (83) were able to use exogenous [$^3$H]thymidine incorporation to measure DNA synthesis after the population had been cultivated for two serial transfers in Hi-glucose medium supplemented with unlabeled precursor. This implies that uptake or incorporation, or both, of specific exogenous precursors is inducible and that the delay in incorporation may be due in part to the need for induction, and possibly to a physiological difference in such inducibility between swarmers and stalked cells.

**Ribonucleic acid synthesis.** RNA synthesis (also measured by precursor incorporation) was reported to fluctuate during the cycle (48). The rate of RNA synthesis was low during the first 30 min (I period), rose to a maximal rate that was sustained from 90 to 160 min (C period), then decreased again at 160 to 180 min as the population approached cell division (D period).

**Synthesis of proteins.** Fluctuations in rate of protein synthesis were reported by Galdiero (76) and by Iba et al. (101), with relatively lower rates being observed during the D period. Agabian et al. (1), in contrast, reported that the specific rate of incorporation of labeled amino acids remained constant throughout the swarmer cycle.

More significant with respect to protein synthesis have been the findings that although the majority of proteins seem to be synthesized in constant balance with each other and to undergo only neglible turnover during the cycle (27, 76, 101), fluctuations in relative rates of specific protein syntheses occur (27, 61, 101, 143, 145, 209, 251). Pulse-labeling experiments to investigate this question have been performed in two ways: (i) by examining the total electrophoretic display of proteins in a constant-volume sample to detect any bands that appear or disappear during the cycle and (ii) by examining the proteins in a constant-radioactivity sample to identify changes in the relative proportions of proteins synthesized during each successive pulse period.

The two methods have yielded significantly different results. When constant-volume samples of synchronous *C. crescentus* CB13 populations were analyzed (101), only one nonflagellar protein (77 kD; flagellar proteins are discussed separately, below) was found to be synthesized periodically; it was not detected until the 75-to-90-min pulse (early C period) of the 240-min cycle, or 0.3 cycle unit, and its synthesis continued through the D period. In contrast, by analysis of samples normalized with respect to radioactivity, as many as 14 (27) to 19 (1) proteins were found to fluctuate in *C. crescentus* CB15 in proportion to total protein synthesized during the successive pulse periods. In the two laboratories pursuing this method, the experiments differed with respect to procedure for preparation of swarmer populations, duration of pulse-labeling periods, identity and concentration of labeled precursors, method for halting labeling, and methods for fractionating cells. These several differences may be partly responsible for the small degree of overlap in their findings (Table 9), so it is not yet possible to focus on any nonflagellar protein(s) as distinc-

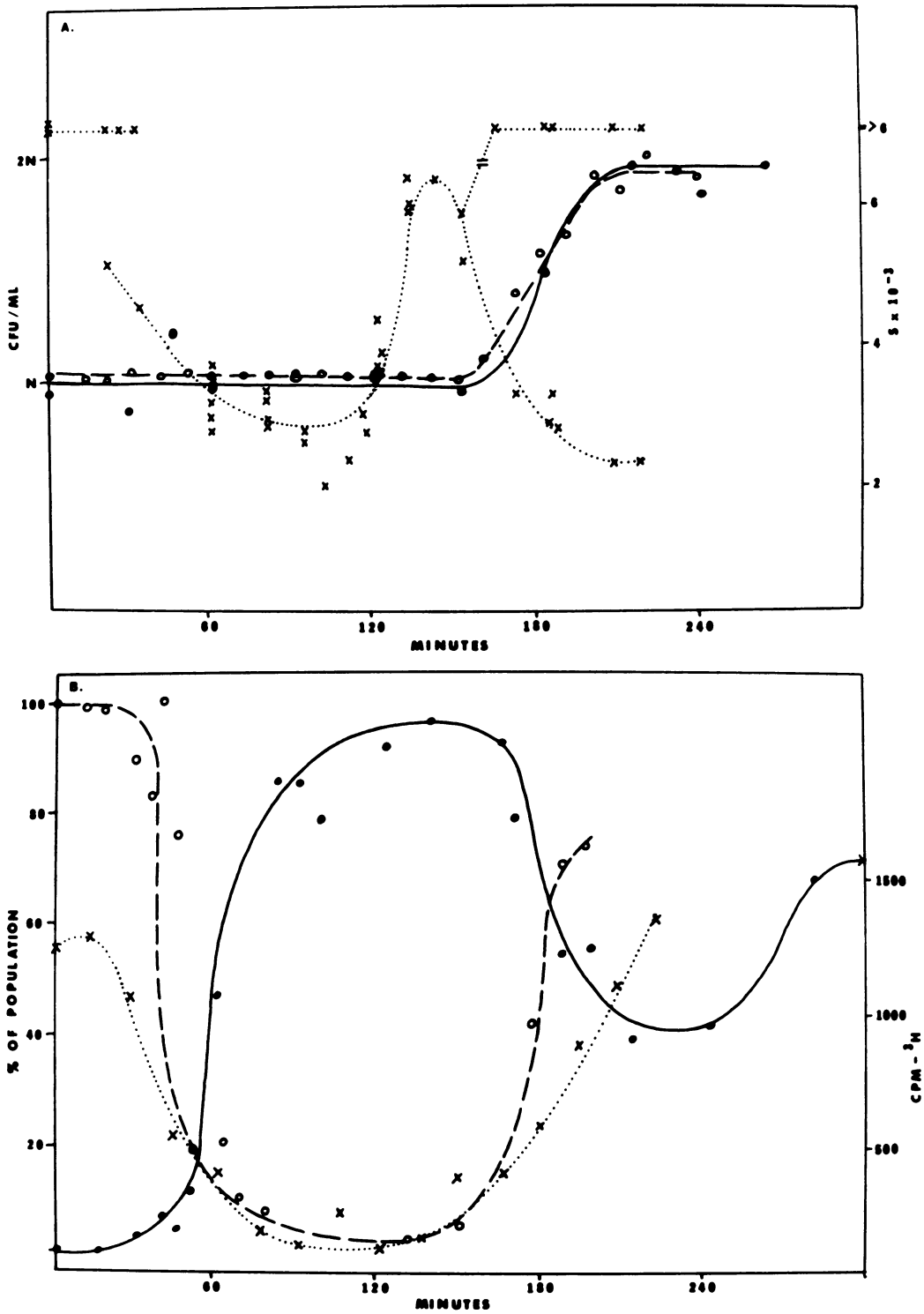


FIG. 6. *Events in synchronous populations of C. crescentus CB15 swarmers in Hp-glucose medium. (A) Cell division and sedimentation behavior of EANs. Symbols:* ○——○, *viable count (48) (n = 1.0 × 10⁷ colony-forming units [CFU] per ml);* ●——●, *viable count (60) (n = 4.2 × 10⁸ CFU/ml,* ×····×, *sedimentation value of EAN fraction (61). (B) Polar organelle development. Symbols:* ○——○, *percentage of population motile (193);* ●——●, *percentage of population stalked (48, 193);* ×····×, *³H-labeled phage φCbK irreversibly adsorbed (60).*

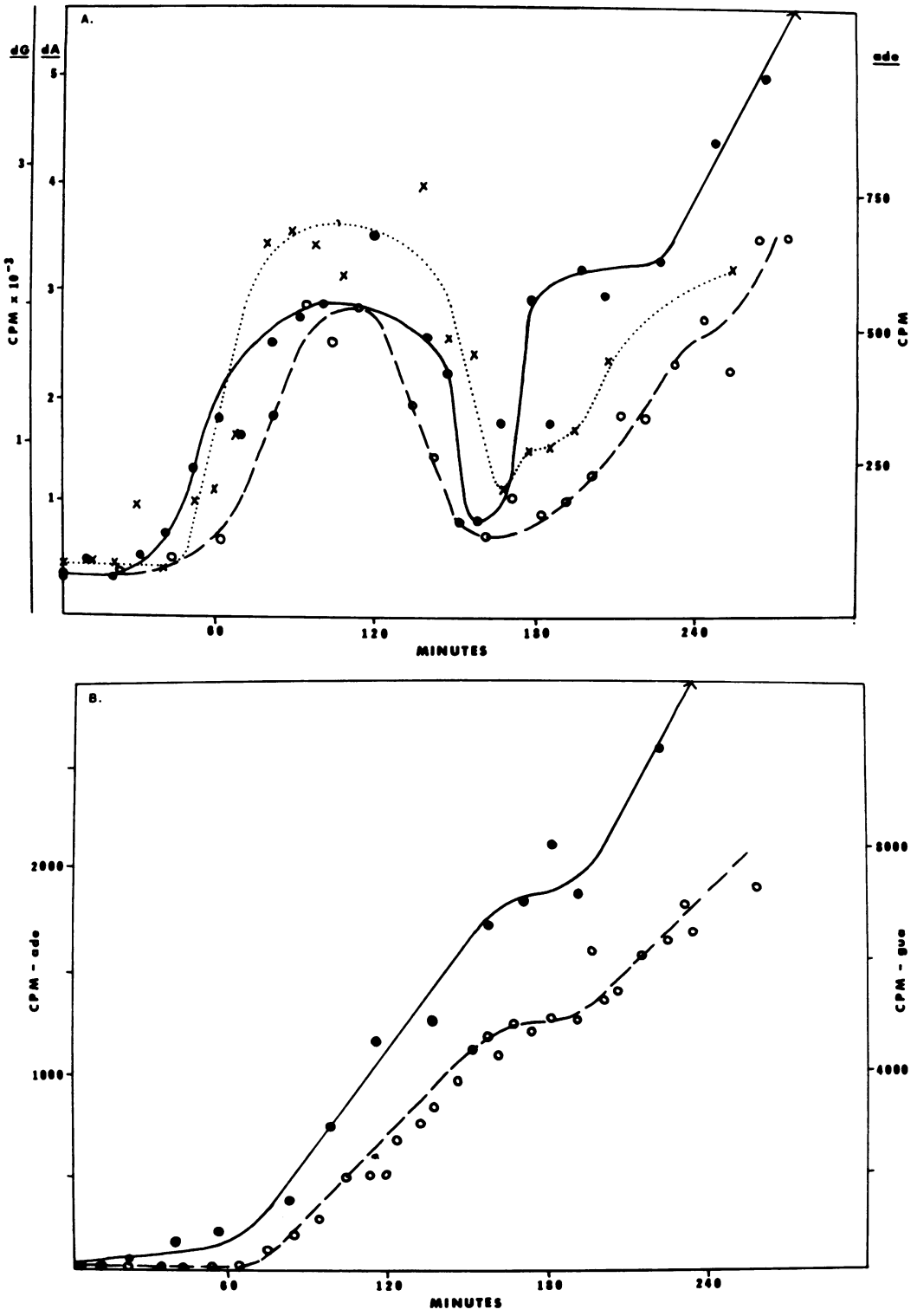


FIG. 7. *DNA synthesis in synchronous populations of C. crescentus CB15 swarmers in Hp-glucose medium (A) Pulse-labeling. Symbols:* O——O, *[³H]deoxyguanosine (dG) incorporation, 8-min pulse (48);* ●——●, *[³H]deoxyadenosine (dA) incorporation, 5-min pulse (48);* ×····×, *[³H]adenine (ade) incorporation, 2-min pulse and 2-min chase (60). (B) Continuous labeling.* O——O, *[³H]guanosine (gua) incorporation (209);* ●——●, *[³H]adenine (ade) incorporation (193).*

TABLE 8. *Cell division and DNA synthesis in synchronous populations of C. crescentus swarmers*

| Strain | Medium[a] | Swarmer selection method[b] | Cell division (min) | | DNA synthesis | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | | Interval[c] | Duration | Precursor provided[d] | Interval (min)[c] | Onset of resumption (min)[c] | |
| CB2 | PYE | Diff. cent., 4°C | 60–120 | 60 | | | | 218 |
| | | Diff. cent., 31°C | 45–105 | 60 | | | | 218 |
| CB13 | PYE | Diff. cent. | 90–110 | 20 | | | | 76 |
| | | Diff. cent. | 95–110 | 15 | | | | 247 |
| | | Elution (7 min) | 80–150 | 70 | dA | 25–80 | 100 | 99 |
| | | Elution (7 min) | 100–140 | 40 | | | | 100 |
| | | Elution (15 min) | 80–105 | 25 | | | | 147 |
| | | Accumulation | 80–120 | 40 | | | | 98 |
| CB13tdr-806B35R4 | Hp-glu | Elution (7 min) | 160–240 | 80 | | | | 101 |
| CB15 | Hp-glu | Elution (10 min) | 150–210 | 60 | dA | 50–150 | 170 | 48 |
| | | | | | dG | 65–145 | 200 | 48 |
| | | Elution (10 min) | 150–200 | 50 | | | | 27 |
| | | Elution (10 min) | 170–210 | 40 | A | 75–160 | 210 | 193 |
| | | Elution (10 min) | 180–240 | 60 | G | 60–150 | 190 | 209 |
| | | Elution (10 min) | 180–240 | 60 | | | | 276 |
| | | Grad. cent. | 160–200 | 40 | A | 60–170 | 190 | 60 |

[a] The exponential generation time for all strains in PYE is ca. 100 min (217, 287); for CB15 in Hp-glucose (Hp-glu), it is 180 min (277). All cultures represented here were incubated at 30°C with mechanical agitation sufficient for adequate aeration and minimal attachment to the vessel walls.

[b] See text for description of methods. Diff. cent., differential centrifugation; grad. cent., Ludox density gradient centrifugation; elution, harvesting swarmers released by attached cells by exchange of medium at interval indicated in parentheses; accumulation, harvesting swarmers and unattached stalked cells by removal of medium from tube culture incubated with minimal agitation.

[c] Time after reincubation of the swarmer population.

[d] dA, [$^3$H]deoxyadenosine; dG, [$^3$H]deoxyguanosine; A, [$^3$H]adenine; G, [$^3$H]guanosine.

tive of a given step in the development sequence. Nevertheless, it is clear that shifts in patterns of protein synthesis occur at two points during the swarmer cycle. A major shift, involving mainly increases in proportional syntheses of several soluble and membrane proteins, occurs at the beginning of the C period concomitantly with the onset of stalk development, DNA synthesis, and expansion of the nucleoid. A minor shift occurs late in the C period; this shift is seen almost exclusively among membrane proteins, and predominantly as an increased proportion of certain proteins. Conceivably, this alteration in profile reflects redistribution or processing of proteins during surface reorganization at the fission site.

A relationship of the second, relatively minor shift in protein synthesis pattern to membrane reorganization is also implied by the findings of Mansour et al. (171) in their studies of patterns of phospholipid biosynthesis. A clear change in pattern occurred at the end of the C period. Up to that time, phospholipid biosynthesis resulted in the accumulation of a variety of substances which were gradually converted to phosphatidylglycerol. The pattern then shifted to relatively rapid accumulation of phospatidylglycerol and a concomitant reduction in the amounts of phosphatidic acid and other, minor phospholipids. After cell division, the stalked sibling apparently continued to follow the second pattern of phospholipid biosynthesis, whereas the swarmer resumed the earlier pattern. In contrast to patterns of protein synthesis (and to phospholipid synthesis in synchronous *E. coli* [217]), however, phospholipid biosynthesis did not change at the onset of the C period.

The possibility of asymmetric distribution of proteins in preparation for cell division is implied by the observations of Agabian and co-workers (1, 61). They studied the distribution of proteins that had been pulse-labeled during only one of the three stages of the swarmer cycle and then chased through the completion of cell division into the respective swarmer and stalked-cell progeny. Evidence of asymmetric distribution of several proteins was detected; the differences related principally to localization within each cell type, among soluble, inner membrane, and outer membrane fractions, and not often to total absence of any protein from one type of progeny. At least seven proteins present in both cell types were localized in the outer membrane of the stalked sibling, but not in that of the swarmer sibling; none of the seven is known to be restricted to the stalk.

Unexpectedly, the respective siblings inherited different arrays of soluble proteins. One or two proteins of approximately 89 to 90 kD and a 63-kD protein were found in the soluble fraction of the swarmer progeny only, whereas only the stalked siblings inherited 42-, 35-, 33-, and

TABLE 9. *Modulation of synthesis of specific proteins in synchronous populations of C. crescentus swarmers*

| Cell fraction | Maximum proportional synthesis[a] during: | | | | |
|---|---|---|---|---|---|
| | Early I | Early C | Middle C | Late C | D |
| Soluble | | **98** | **98** | **98** | **98** |
| | | **88** | **88** | **88** | **88** |
| | | **64** | **64** | **64** | **64** |
| | | 61 | 61 | 61 | 61 |
| | 57 | 57 | 57 | 57 | |
| | | **56, 56** | **56, 56** | **56, 56** | **56, 56** |
| | | **33** | **33** | **33** | **33** |
| | **29** | | | | |
| | 28 | | | | |
| | **24** | | | | |
| | 20 | | | | |
| Envelope | | | | | |
| Outer membrane | **108** | **108** | | | |
| | **95** | | | | |
| | | **94** | **94** | **94** | **94** |
| | | | | **91** | **91** |
| | **88** | | | | |
| | | **87** | **87** | **87** | **87** |
| | 85 | | | | 85 |
| | | | | 75 | |
| | | | | 69 | |
| | | | | 65 | 65 |
| Inner membrane | | **70** | **70** | | |
| | | 67 | 67 | 67A | 67A |
| | | | | 67B | 67B |
| | | | | | 48 |
| | 44 | | | 44 | 44 |
| | **43** | **43** | **43** | **43** | |
| | | 23 | 23 | | |
| Membrane | | | | 135 | |
| | | | 62 | | |
| | | | | | 59 |
| | | 53 | | | |
| Flagellum | | | | | |
| Major flagellin | +, + | + | +, + | +, + | +, + |
| Minor flagellin | | | +, + | +, + | +, + |
| Hook monomer | | | + | + | + |

[a] Results of studies by Agabian and co-workers (1, 145) are in boldface; results of Newton and co-workers (27, 209; M. J. Clancy, and A. Newton, Abstr. Annu. Meet. Am. Soc. Microbiology K170, 1978, p. 155; M. J. Clancy and A. Newton, personal communication) are in lightface. Numbers represent the estimated sizes (in kilodaltons) of distinguishable proteins.

21-kD proteins as soluble components. These observations imply considerable compartmentation within the cell long before cell division, since the patterns of asymmetric distribution of soluble as well as outer membrane proteins were observed even when the pulse had occurred during the I period of the cycle; i.e., soluble proteins synthesized as early as the swarmer stage were asymmetrically distributed to the progeny 150 min later.

In similar pulse-chase experiments in which proteins of the EANs were examined before the chase period and again after cell division (61), at least two proteins (70 and 62 kD) were more abundant in the EANs of the stalked cell progeny, whereas smaller proteins (26, 19, and 15 kD) were found only in the EANs of swarmer progeny. Of these, the 70- and 26-kD species were not envelope proteins; the 62-kD protein is possibly identical with one of the soluble (calf-thymus-)DNA-binding proteins detected by Cheung and Newton (27).

One specific set of proteins has been amenable to more concrete analysis, since their role in the differentiation cycle and their function in the cell are known; these are the three flagellar proteins flagellin A, flagellin B, and hook protein. During the uninterrupted developmental cycle, flagellin A was synthesized in the early I period, but its relative synthesis decreased to negligible in the late I and early C periods. Synthesis once again became detectable in mid-

C (0.6 to 0.67 cycle unit) (1, 145, 209, 251), accompanied by synthesis of flagellin B (145, 209) and hook protein (145); synthesis of all three proteins accelerated rapidly at the conclusion of the C period. Both flagellins were assembled into shear-sensitive filaments from the time of acceleration of synthesis; approximately one-half of the flagellins synthesized at any time remained associated with the envelope after shearing, and only the minor flagellin (B) was found in measurable amounts in the soluble fraction (145). After cell division, synthesis of flagellin B and hook protein rapidly returned to an undetectable proportion, whereas flagellin A synthesis persisted for about 20 min. The continued synthesis of flagellin A was attributed to a relatively long-lived flagellin A messenger RNA, since rifampin did not affect the rate or duration of synthesis (1, 209).

In contrast to the behavior of flagellar protein synthesis during the uninterrupted swarmer cycle, Osley et al. (209) have found that interference with DNA synthesis by (bactericidal) chemical inhibition or transfer of thermosensitive DNA mutants to the restrictive temperature prevented detectable synthesis of the flagellins. In the thermosensitive mutants, the effect was reversible, but the onset of flagellin synthesis upon return to the permissive temperature was delayed until the resumed round of DNA synthesis was completed. Osley et al. interpreted this as evidence that completion of DNA synthesis was required for the transcription of the flagellin genes. However, since completion of DNA synthesis was not required in the uninterrupted cycle, it seems at least equally reasonable to propose that transcription (or translation) recovered more slowly than DNA replication.

Further, since constriction, which normally begins before completion of DNA synthesis (195, 276), was also suspended at 37°C, the regulation of flagellin synthesis appears more closely related to surface events than to DNA synthesis. The following proposal offers a means of reconciling the results of the two types of studies on flagellin synthesis, viz., that a surface event in preparation for cell division is required for organization of a flagellum assembly site. Once available, this site would allow removal of flagellin proteins in the assembly of the external filament, thereby relieving product inhibition of flagellin synthesis. On the basis of this proposal, it would be predicted that after cell division, flagellin synthesis would continue only in the swarmer and not in the stalked sibling, as has already been observed (1, 209). It further predicts that interference with flagellar assembly would reduce flagellin synthesis, a phenomenon seen in the presence of the polyhook mutation (254). Recent studies on interference with phospholipid synthesis by cerulenin inhibition or by glycerol deprivation of a glycerol auxotroph (34, 36) imply that preparation of the site is dependent on normal phospholipid synthesis.

Although this proposal, by interposing another (hypothetical) site, may seem to beg the question of whether completion of DNA synthesis can directly trigger acceleration of flagellin synthesis, it could redirect experimental attention away from flagellin synthesis, which may be secondarily affected, and stimulate postulation and recognition of the surface event whose occurrence is directly signaled by DNA completion. That event might be common to reproduction of gram-negative bacteria in general.

Since none of the asymmetrically inherited proteins other than flagellins is known to be synthesized only late in the cycle, the results of the pulse-chase studies imply that protein composition is asymmetric during most of the cell's growth. Although a large division site mesosome is typical of sectioned *Caulobacter* cells (223), this structure does not clearly separate even the late-cycle cell into two discrete cytoplasmic units. Thus, the outward manifestation of asymmetric construction, with one pole stalked and the other eventually flagellated, must reflect a higher degree of internal asymmetry than could have been anticipated or can yet be accounted for by observations of internal organization.

**Nonintermittent events.** Events that do not fluctuate during the cycle include generalized cellular growth, measured as rate of increase either of turbidity (247) or of cell volume (276), synthesis of pilin (143) and of DNA-dependent RNA polymerase (27, 61, 101), rate of synthesis of polyadenine RNA (200), association of the nucleoid with the cell membrane (61), and stability of the majority of cellular proteins (27, 61, 101).

### Mutations Affecting Cell Division

One of the most illuminating groups of mutants of *C. crescentus* yet reported comprises 10 thermosensitive mutants isolated by Osley and Newton (206; see also references 195 and 207 through 209). The properties of these mutants and of thermosensitive motility mutants are presented in Table 10. In the table, the first five developmental events have been arranged in an order intended to imply dependence of an event to the right on the events to its left. The properties of the thermosensitive mutants imply that the capacity for DNA synthesis is necessary for constriction site organization and flagellin synthesis (206) and distantly necessary, but not

TABLE 10. *Thermosensitive (ts) properties of conditional cell division and motility mutants of C. crescentus*[a]

| Strain | Capacity at 37°C[b] for: | | | | | | | Colony formation |
|---|---|---|---|---|---|---|---|---|
| | Cell division mutants | | | | | Motility mutants | | |
| | DNA initiation | DNA elongation | Constriction | Stalk initiation[c] | Cell separation | Flagellin synthesis | Flagellum assembly | |
| CB15 | | | | | | | | – |
| PC2076 | | | | | | | | |
| round 1 | | + | + | ND[d] | + | + | + | |
| round 2 | ts | (–) | – | – | (–) | – | (–) | |
| PC1042 | NT[e] | ts | – | – | (–) | – | (–) | – |
| PC1049 | + | + | ts | – | (–) | + | + | – |
| PC1040 | + | + | + | + | ts | + | + | – |
| CB13 | | | | | | | | |
| AE1 | + | + | + | + | + | ts | (–) | + |
| AE5 | + | + | + | + | + | + | ts | + |

[a] Properties of PC strains as described by Osley and Newton (206; see also references 195, 207, and 209) and strains AE1 and AE5 as described by Marino et al. (172).

[b] Parentheses indicate any process dependent, a priori, on another process, e.g., flagellum assembly's dependence upon flagellin synthesis.

[c] Stalk initiation at a pole other than a pole formed and flagellated at the permissive temperature (30°C).

[d] ND, Not described.

[e] NT, Not tested; initiation was determined by measuring incorporation of exogenous precursors into polymer, so the first step could be detected only if polymer elongation followed.

sufficient, for stalk initiation. The capacity for constriction is necessary for stalk initiation, but not for flagellar assembly. Thus, each of the morphogenetic events emerges as a process that is to some extent dependent on the capacity for DNA synthesis or on another morphogenetic event but that is also independently determined. It is not, therefore, surprising that morphogenetic mutants comprise a mixture of types altered in a single trait and others that exhibit multiple changes.

In Osley and Newton's most recent analysis of their thermosensitive mutants (208), the mutants were subjected to successive inhibitions by temperature and by antibiotics. In this way, Osley and Newton were able to identify at least one thermosensitive process with the process inhibited by hydroxyurea and to demonstrate that the morphogenetic processes are dependent on continued DNA synthesis. Viability was not determined. Contreras et al. (36) have simultaneously demonstrated the dependence of DNA synthesis on continued membrane biosynthesis. The implication of these two sets of studies taken together is that surface extension and DNA synthesis are mutually dependent processes in *C. crescentus* under the conditions used in these studies and that artificial interruption of either process may lead quite rapidly to cessation of the other.

## Inhibition of Morphogenesis

The development of the caulobacter stalk is clearly a process dependent on growth and predictably should be inhibited by agents that reduce any process required for general cellular growth. J. M. Schmidt (Ph.D. thesis, University of California, Berkeley, 1965) found stalk development susceptible to inhibition by actinomycin D, puromycin, streptomycin, and chloramphenicol. In later reports on the effects of a variety of inhibitors used to interfere with specific biosyntheses in bacteria, their respective effects have been found less specific in *C. crescentus* than anticipated; these agents have included rifampin (193), hydroxyurea (93), cerulenin (36), and nalidixic acid, phenylethyl alcohol, and phleomycin (47).

Only three agents have been found usefully specific for one cellular process: mitomycin C (83), penicillin G (277), and *cis*-platinum(II) diamminodichloride (whose effect was described above [183]), each of which, when used at subbactericidal concentrations, inhibited cell division while allowing continued synthesis of cell components in near normal proportions. In the presence of any of these substances, *C. crescentus* cells grew as unconstricted filaments. In mitomycin C-induced filaments, the DNA content was slightly (less than 20%) reduced, and the nucleoplasm was not so readily detectable as in sections of uninhibited cells. Reversal of the inhibition was not described. The addition of penicillinase to penicillin-induced filaments resulted in a resumption of cell division within 60 min. The nonstalked cells produced from filaments averaging 7 to 8 μm in length were 3 to 5 μm in length, indicating that fission was binary

and roughly equal despite the excessive length of the filaments. Like normal swarmers under favorable conditions, the nonstalked progeny proceeded to develop stalks before their first cell division, which was not, however, synchronous; once begun, cell multiplication proceeded without arriving at a plateau (277).

The inhibitory effects of mitomycin C and penicillin occurred only after a 30- to 60-min delay, and stalk initiation occurred in swarmer populations. Stalk elongation, in contrast, was inhibited by both agents, so that the filaments had stalks that were short in proportion to filament length. Motility was absent, even though the penicillin-induced filaments assembled at least one flagellum on the nonstalked pole. Both groups of investigators (83, 277; see also reference 195) concluded that either stalk formation was dependent on the completion of cell division or the site of action of the drug was common to both types of surface morphogenesis—cell division and stalk development.

In the nutrient-poor aquatic environment that is the natural habitat of caulobacters, lack of nutritional support rather than specific inhibition must be dealt with by the cells. DNA synthesis, for example, probably occupies only a small fraction of the lifetime of any individual cell, occurring only in preparation for imminent reproduction. Accordingly, close coordination between DNA synthesis and surface events, such as constriction and flagellar activation, would serve to condense reproductive activities into periods during which there was adequate nutritional support for population expansion. The influence of nutrient supply on initiation and progress of DNA synthesis and coordinated events is yet to be explored. Developmental studies with nongrowing populations of caulobacters have so far received scant attention, even though marginal growth conditions are generally known to result in relatively elaborate stalk development. It seems possible that although the sequence of several events appears invariable in steadily growing populations, alternative developmental patterns and regulatory processes may operate under conditions that support morphogenesis in the absence of constant cellular growth and regular rounds of reproduction.

## Prospects in Molecular and Genetic Analysis of Development

**Transcription.** Like other bacterial RNA polymerases, the *C. crescentus* enzyme consists of four subcomponents: $\beta'$ (165 kD), $\beta$ (155 kD), $\sigma$ (96 to 101 kD), and $\alpha$ (44 to 48 kD) (7, 17, 28). The core and $\sigma$ subcomponents have been resolved by DNA-cellulose chromatography (17, 28). The $\sigma$ subunit was not active with core enzyme in reconstituting the holoenzyme (28), but *E. coli* $\sigma$ factor reconstituted the activity and the specificity of *C. crescentus* core polymerase (7). Heterologous DNAs, e.g., from coliphage and calf thymus, can serve as templates (7, 17, 104, 284). As in several other bacteria, the *C. crescentus* (CB13) polymerase is capable of rifampin-sensitive unprimed synthesis of polyadenylate (28) and of polyadenylate-polyuridylate (104); unlike such synthesis by the *E. coli* enzyme, unprimed synthesis was highly specific for uridine triphosphate and adenosine triphosphate as substrates, and cytidine triphosphate and guanosine triphosphate did not interfere with the reaction.

The principal question regarding the polymerase is whether it varies in structure, specificity, or activity during the developmental sequence. RNA polymerases isolated from each of the three periods of the swarmer cycle were found to be identical in subunit composition and to exhibit comparable activities with phage T2 DNA as template (17). The activity of the polymerase in polyadenylation of RNA (28) and the probable location of the polyadenylate tracts on small, relatively short-lived (15- to 20-s half-life) RNA molecules (101, 200) suggest that a special subset of messengers may be produced by the enzyme. It seems unlikely that this subset could exert immediate and direct control over the sequence of developmental events in caulobacters, however, since the rate of polyadenylated RNA synthesis did not vary during development (200), and the effect of rifampin on each affected event was considerably delayed (by 10 to 30 min [193]) relative to the half-life of the polyadenylated RNA.

At present, the most important observation with regard to the possible central role of RNA polymerase in the discontinuous expression of genetic information in caulobacters is the strong evidence that there is a single species of this enzyme (17, 200). Accordingly, discriminatory transcription during the morphogenetic sequence cannot be explained by differentiation of the polymerase and will have to be accounted for by other molecular mechanisms.

**Translation.** In vitro translation by *C. crescentus* ribosomes and of caulophage RNA has been achieved (68, 155–157, 256, 273, 274), and the procedures for preparation of active systems have been found to be essentially the same as those used with *E. coli*. Some evidence of species specificity for messenger RNA was inferred from the inability of *C. crescentus* ribosomes to bind or translate coliphage MS2 RNA, whereas *E. coli* ribosomes were unable to bind and only

weakly translated caulophage φCb5 RNA (155, 156). It is possible that some of the apparent specificity was due to the presence of inhibitory factor(s) in φCb5 RNA preparations, although virions can be prepared without cell disruption during prelytic phage release (154). The RNA from phage φCp2 was reported to be inert in an *E. coli* translation system unless it was purified by benzoylated, naphthoylated diethylaminoethyl cellulose chromatography (69).

Ribosomal factors IF-2, IF-3 (24 kD [155]), and S1 (67 kD [278]) prepared from *C. crescentus* are interchangeable with the corresponding factors from *E. coli* (155, 156, 273, 278), although IF-1 of *C. crescentus* cannot be removed from the ribosomes so readily as it can from *E. coli* ribosomes (157).

Although the in vitro studies have not provided any recognizable clues to the mechanism by which major regulatory components may influence development in caulobacters, they are reassuring in the sense that the caulobacter system presents itself as one amenable to exploration of the molecular details of its cellular control mechanisms. The close similarity between *C. crescentus* and *E. coli* systems encourages the expectation that the wealth of information concerning regulation of gene expression accumulated from studies with *E. coli* will apply with minimal modification to *C. crescentus*.

**Conjugation.** Genetic transfer dependent on cell contact was first studied by C. Ruby (M.S. thesis, Indiana University, Bloomington, 1967) in derivatives of *C. crescentus* CB15. The recombinant frequencies observed for auxotrophic and drug resistance markers ranged from $10^{-7}$ to $10^{-5}$ recombinant per donor, against a mutation frequency of $10^{-8}$ or lower. Recombinant frequencies were as high in matings in broth as in matings on agar. Cell contact was required; transfer was not mediated by culture filtrates, nor did it occur between strains growing in a modified U-tube designed for cultivation of strict aerobes in chambers separated by microfilters.

Shapiro et al. (248), Jollick and co-workers (115, 116), and Newton and Allebach (194) have also reported conjugal transfer among CB15 and its derivatives. Generally, recombinant frequencies have been about $10^{-5}$, although Newton and Allebach observed some in excess of $10^{-2}$. The transfer seemed dependent on incubation of the mating populations on agar medium (either nutritive or selective), implying that transfer was slow and required prolonged contact (or cell lysis [194]) and that motility, retarded by agar, was not important in the establishment of effective contact.

In a dimorphic bacterium, the question of whether one or the other form is more active as a conjugal donor seems valid, and the swarmer has been suggested as the donor stage (6, 195, 248). It seems particularly relevant to *C. crescentus* CB15 conjugation, since evidence of strict polarity of transfer has not been obtainable. Jollick and Tran (116) observed that in crosses of *C. crescentus* CB15/WS12, recombinant formation was eliminated by streptomycin in crosses with WS12 $Str^s$, but not with WS12 $Str^r$. They inferred that WS12 behaved as a recipient in their crosses, but the inference was weakened by their further observation that the presence of streptomycin in the mating broth or on the selective plating medium resulted in at least a 10-fold reduction in recombinant frequency even when the growth of both partners was streptomycin resistant.

Newton and Allebach (194) identified 3 strains as "fertile" and 12 as "nonfertile" on the basis of higher and lower recombinant frequencies. In crosses between fertile and nonfertile strains, unselected drug resistance markers (*str* and *rif*) among prototrophic recombinants were more often inherited predominantly from the nonfertile partner. In some crosses, however, polarity could not be discerned, and in more than one cross polarity appeared reversible and dependent on the drug resistance marker assayed.

Since each of the 12 nonfertile strains was able to form recombinants with other nonfertile strains, it is possible that the difference between the fertile and nonfertile strains lay in the ability of the former to assume both donor and recipient roles with any other strain, whereas lower recombinant frequencies resulted from matings between strains in which the partners were restricted to only one type of interaction. The low frequency of prototrophic recombinants with the four mutually sterile $His^-$ and $Met^-$ strains studied would be explained if they usually behaved only as recipients, and their *his* and *met* markers were transferred late. A mechanism for mutual conjugal exchange would seem appropriate for bacteria that live in sparsely populated communities; the caulobacters may have evolved a means of procaryotic hermaphroditism.

Alexander and Jollick (6) succeeded in transfering RP1 ($Tet^r$ $Kan^r$ $Car^r$ phage $PRR1^s$), a *Pseudomonas* plasmid, from *Pseudomonas aeruginosa* PAO67 into *C. vibrioides* Cv6 and *C. crescentus* CB15/WS30 (a *met* auxotroph). All three of the drug resistance markers of RP1 were expressed in Cv6. Sensitivity to phage PRR1 was not expressed, but the phage sensitivity marker could be transferred from Cv6 $R^+$ into *E. coli* CSH29. Some indication of chromosome mobilization in Cv6 was observed, as

transfer of *met*$^+$ from Cv6 R$^+$ ($B_2$ requiring) to WS30; however, the $B_2$-independent *met* prototrophs were not stable.

Ely (57) reported transfer of RP4 (not known to be different from RP1 [42, 201]) from *E. coli* J53(RP4), as well as four other plasmids, into *C. crescentus* CB15. The transfers, carried out on membrane filters, occurred at frequencies up to 100% of the recipient population in 2 h. Transfer from *C. crescentus* (RP4) to *C. crescentus* or *E. coli* was about 10-fold less efficient. *C. crescentus* (RP4) strains exhibited resistances to the antibiotics, and they absorbed, but were not productively infected by, phage PRR1. They also exhibited generation times 50% longer than those of plasmid-free strains. In crosses between R$^+$ and R$^-$ CB15 strains, mobilization of the *C. crescentus* chromosome was detected as formation of prototrophic or drug-resistant (Str$^r$ Rif$^r$) recombinants at frequencies of $10^{-8}$ to $10^{-6}$. Examination of unselected markers revealed that in two of the three crosses, the RP4-carrying strain had behaved as donor. In the exceptional cross, the apparent direction of polarity depended on the identity of the markers.

Although RP plasmid-mediated transfer results in recombinant frequencies lower than spontaneous mutation rates (estimated as $10^{-7}$ to $10^{-6}$ [109]), and much lower than recombinant frequencies even among "nonfertile" strains (more than $10^{-4}$ [194]), it confers fertility on otherwise sterile crosses (57). It is possible that such plasmids will confer conjugative capacity on strains other than *C. crescentus* CB15, the only strain so far found capable of unassisted conjugal transfer and the only strain susceptible to the transducing phages $\phi$Cr30 and $\phi$Cr35 (59, 112).

## Summary

The diagrammatic representation of the *Caulobacter* cell cycle presented several years ago (218), in which the relationships among the three basic morphological forms that occur during the cycle were indicated, can now be recognized also as a representation of regulatory changes during the three cycle periods: I (motility), C (elongation—of cell, stalk, and DNA), and D (reproductive). The first period can now be viewed as a stage of limited biosynthetic activity; at its conclusion, the synthesis of DNA and of several specific proteins is initiated. The second period is the most active with respect to both morphogenesis and biosynthesis; during this stage, stalk development and, later, flagellar syntheses accompany DNA replication and general cellular growth. The final stage involves rearrangements of the nucleoids and the cell surface and assembly of the flagellar apparatus, and presumably differentiation of the regulatory metabolism so that, upon completion of cell division, different biosynthetic patterns will be expressed in the respective siblings. Thus, each stage presents a specialized condition that can be explored as a differentiated state in itself or as a bridge linking the other two conditions. In this respect, investigations of synchronous populations of caulobacters are proving a highly tractable system for the investigation of procaryotic cellular differentiation in a continuously vegetative state. Elucidation of the interaction between genes that govern structural organization and their target organelles (in this case, the cell envelope and its appendages and extrusions) should carry implications regarding developmental processes in other bacterial systems, as well as in organisms that seem remote from prosthecate bacteria.

## ECOLOGICAL IMPLICATIONS

The ubiquity of caulobacters, principally *Caulobacter*, in nutrient-poor habitats and their corresponding absence from nutrient-rich environments, such as living tissues and decaying animal and vegetable matter, are the principal characteristics of their natural distribution. In this respect, they are joined among morphological types of chemoheterotrophic bacteria only by *Hyphomicrobium*. Rods, spirilla, vibrios, both mycelial and nonmycelial sporulating forms, cocci, and spirochetes—even other prosthecate bacteria, such as *Prosthecobacter*, *Prosthecomicrobium* and *Ancalomicrobium*—exhibit no such wide and at the same time strongly skewed distribution corresponding to the ambient concentration of organic nutrients. Since most of the biosphere is watery and low in organic content, spattered with islands of organic abundance, the preferred habitat of *Caulobacter* is a major fraction of the inhabited regions of this planet.

The terms *oligotrophic* and *oligocarbophilic* have been used to designate organisms whose development and survival as vegetative populations are favored by low concentrations of organic substrates. I propose the term *copiotrophic* for microbial types whose survival seems dependent on a nutrient supply that is typically 100 times higher than that found in oligotrophic habitats. Nearly all the bacteria that have served research in bacterial physiology for the past century have been copiotrophic. Oligotrophy has not been distinguished from copiotrophy in specific physiological terms, and the present designation of a given isolate as oligotrophic is usually based solely on its ability to produce visible growth on media containing

only 5 to 10 mg of carbon per liter.

The question of whether any bacteria could survive in nature as obligate oligotrophs, unable to take advantage of occasional, transient periods of nutrient enrichment, is dealt with elsewhere for bacteria in general (89, 141). In the following discussion, attention will be directed toward four questions relating to the peculiar distribution of *Caulobacter* and the less common *Asticcacaulis*. Too little fundamental physiological work has yet been carried out with caulobacters to allow confident conclusions. Consequently, this discussion consists largely of speculations, not conclusions, and is offered in the hope that it will point out some of the major physiological studies whose pursuit would contribute to a firmer foundation for the interpretation of the ecology of this group.

## Questions

**Question 1: nutrient sensitivity.** Are growth and multiplication of caulobacters inhibited by elevated nutrient concentrations, and, if so, are caulobacters especially sensitive to higher concentrations of particular nutrients? Although this question has not been systematically explored even with those caulobacters that can be cultivated in defined media, sensitivity to moderately high concentrations of nutrients does not seem specific for any one type of substance. Complex nutrients (12, 14, 15, 80, 93, 97), glucose (152, 160), amino acids and nonnitrogenous organic acids (218), phosphate (152; Poindexter, unpublished data), and other ions (82, 152, 218) all seem to have low upper limits allowing uninhibited growth and uniform morphology. In *C. crescentus* in *A. biprosthecum*, slowly utilized sugars, such as lactose, can, however, be provided at higher (gravimetric) concentrations than glucose (137, 140, 152).

Nutrient concentration sensitivity in caulobacters is probably related to nutrient balance, not just to the concentration of a specific substrate. This is suggested by the studies of Grula et al. on mineral requirements (82) and of Ely et al. (58) on amino acid requirements among auxotrophic mutants of *C. crescentus*, as well as by the increased tolerance of *C. crescentus* toward glucose in glutamate-supplemented medium (222). If this is the case, testing the effect of various concentrations of single nutrients will be misleading, and the appropriate experiments become unattractively complicated.

The tentative answer to the first question is Yes, and "dilute medium" must be regarded as referring to all ingredients.

**Question 2: reproductive rates.** Is the relative success of populations of caulobacters due to maintenance of near maximal reproductive rates at nutrient concentrations so low that the growth rates of copiotrophic aquatic bacteria are significantly reduced? Growth rates have not been determined for caulobacters in steady-state systems. Accordingly, comparisons of their growth rates with those of isolates of different physiotype, as carried out with nonattaching bacteria (135, 176, 177, 255, 281), are not available.

In dilute media, such as 0.01 to 0.05% peptone used in enrichment and isolation procedures, caulobacters exhibit generation times of 2 to 5 h. Generation times are not reduced by cultivation in media that support significantly higher growth yields. Caulobacters are invariably accompanied in natural samples and in enrichment cultures by bacteria—principally the fluorescent group of *Pseudomonas* in water samples—that exhibit generation times of 1 to 2 h on the isolation media. The fluorescent pseudomonads are not considered oligotrophic and are readily cultivated on such media as undiluted nutrient broth and agar, in which caulobacters generally do not develop. Thus, reproductive rates of caulobacters are lower than those of copiotrophic, nonprosthecate pseudomonads from the same natural sources—in both dilute and richer media. This implies that differential reproductive rate is not an adequate explanation for their higher (sometimes even predominant) proportions in dilute environments.

The other obvious alternative is that their competitive advantage lies not in multiplying more rapidly, but in dying slower. That is, in low-nutrient environments or periods, the caulobacters possibly maintain a relatively stable viable population, whereas other, more "vigorous" types decline (43, 228). The absence of significant decline could more than compensate for a lower rate of multiplication in an environment in which the nutrient supply is meager most of the time.

**Question 3: metabolic regulation.** Do caulobacters exhibit unusual mechanisms of metabolic regulation that are advantageous for growth in low-nutrient-concentration environments? The studies of metabolic regulation so far available have focused principally on sugar uptake and catabolism. Three observations probably significant with respect to this question have been reported. First, although the synthesis of enzyme systems for catabolism of sugars is typically inducible, "induced" levels are not impressively higher than "uninduced" levels for the majority of sugar substrates. The quantitative response of the caulobacter cell to the sudden availability of a new substrate is moderate

in comparison with the magnitude of the response of, e.g., *E. coli* to lactose. In the case of an enteric bacterium, a strong quantitative response to lactose would seem appropriate, since it would serve to support development of the population upon transfer from an adult to the intestine of a newborn mammal. In a dilute aquatic environment, in contrast, the added nutrient in all probability would be available only for a short time, and a maximal shift in biosynthesis could be maladaptive. Instead, in an environment in which practically every utilizable carbon source is available only in small amounts at irregular intervals, maintenance of a significant level of a variety of catabolic systems would allow the cell to consume transient nutrients more readily.

Second, catabolite repression by glucose could not be detected in *A. biprosthecum* (152), although it was inferred for *C. crescentus* (249). Again, catabolite repression is a type of regulation that would not be compatible with survival in an environment where maintenance of a population could not depend on the supply of a single nutrient, no matter how efficiently that substrate could be utilized when available in significant amounts.

Third, the uptake systems (at least in prosthecae) were found to be of low substrate specificity (153). This would seem to allow economical biosynthesis of versatile transport systems suitable for uptake of nutrients available one by one at low concentrations. Competitive inhibition of nutrient uptake would occur during excessive nutrient enrichment, a situation for which caulobacters may be unprepared.

**Question 4: role(s) of the *Caulobacter* stalk.** Does the *Caulobacter* stalk contribute to survival in low-nutrient-concentration environments, and if so, is its contribution physiological, mechanical, or both? When working with caulobacters in laboratory cultures, the mechanical effect of the stalk of an unattached cell soon becomes evident: viz., its drag on the cell when the cell is suspended in liquid. Cells with long stalks do not pack as pellets in centrifugal fields (up to 39,000 × *g* for 60 min) that are suitable for dense packing of the nonstalked cells in the same populations. In wet mounts, unattached long-stalked cells become oriented in microcurrents with each stalk downstream from its cell; when such a cell collides with an object so that it is temporarily reoriented across the current, its progress is greatly retarded until it is once again turned lengthwise by the current. In nature, these mechanical effects are probably responsible for the often-repeated observation that caulobacters are more numerous at the air-water interface than below the surface.

Isolated prosthecae were found to contain some elements of the respiratory and citric acid cycle systems and respiratory energy-dependent uptake systems and to lack the capacity for initial catabolism of sugars and amino acids (122, 153, 226). The attached appendage is biosynthetically active only in the region of its juncture with the cell body (239). These traits imply that the caulobacter prostheca may serve as a means of extending the cell surface, permitting a higher ratio of absorptive surface to metabolically active cytoplasm. The increase in surface/volume ratio achieved by cells growing in laboratory media is not significant unless the media are diluted (20, 78, 93, 106, 131, 152, 160, 161, 218). However, in natural materials, in enrichment cultures, and in association with algal cells (24, 80, 291), stalks of *Caulobacter* cells are not uncommonly 10 times or more the length of the cell body, and cells in nongrowing, nonmultiplying populations are able to develop a second stalk at the outer pole of the cell. Assuming constant distribution of stalk transport activities along its length, this stalk of natural length provides the cell with more than three times the uptake capacity of the surface of the cell body. Translocation of nutrients from the appendage to the cell body has not yet, however, been assessed.

Developmental studies have so far focused on events that occur in nutrient environments that allow unlimited growth relative to the single reproductive cycle usually studied. Even under such conditions, there is evidence that a general biosynthetic retardation occurs during the D period, detected as decreased assimilation of exogenous precursors into cellular macromolecules. After cell division, the swarmer is known to enter into a period of reduced biosynthetic activity even in the presence of nutrients adequate for further reproductive cycles, whereas the stalked sibling rapidly resumes active biosynthesis. However, when the nutrient supply is exhausted, as in stationary phase in closed-system cultures, the swarmers proceed to develop into stalked cells, whereas the stalked cells direct their remaining growth activities principally toward elongation of the stalk. In this way, both progeny appear programed toward prosthecal development under conditions of nutrient limitation of growth. In the natural, oligotrophic environment, such behavior would prevent entry of the cells into a reproductive cycle during any period of nutrient availability below the level adequate to support the completion of the cycle.

Asymmetric cell division, resulting in swarmer production, can be interpreted in a classical

sense, recognizing the motile sibling as a dispersal stage. In the case of caulobacters, there are growing hints that the swarmer may serve to disperse more than just the bacterial population. The possibility that many caulophages preferentially infect the swarmer stage implies that the swarmers also capture phage virions and propagate them. The advantages of bacterial virus production other than genetic exchange are still obscure, but the advantage of refractoriness of the sessile members of the community to virus infection is obvious. In addition, since swarmer production occurs only in growing populations, susceptibility to swarmer-specific viruses would be characteristic only of populations of hosts that were themselves multiplying.

The swarmer has also been suspected of serving as the donor during conjugation (6, 195, 248; but see also reference 143), principally because it is more often piliated. However, since DNA synthesis cannot be detected in motile cells, the swarmer may serve not only as conjugal donor, but also first as recipient; like the bee defended by Anna's king, it may move from flower to flower. Behavior of the swarmer as a genetic vector might account for the peculiar results obtained in reciprocal crosses intended to determine polarity of conjugal transfer in caulobacters. As in the case of virus propagation, the advantageous times for exchange of chromosomal genes would be those periods of nutrient sufficiency when sessile cells can produce both DNA and swarmer siblings.

Less debatable contributions of the caulobacter prostheca to survival are related to the property of adhesiveness. The advantages of stable association with submerged surfaces can be ascribed, for periphytic bacteria in general, to the phenomenon of solute adsorption (174, 292). Attachment of caulobacters appears to be promoted by motility, possibly assisted by pili and finally stabilized by holdfast material. In *Asticcacaulis*, none of this involves the prostheca. In the far more widely distributed *Caulobacter* type, however, lasting attachment is by means of holdfast material on the tip of the stalk. A possible advantage of stalk-mediated attachment could be to remove the cell body from the crowd that develops as the biofilm on the submerged surface. The stalked cell would then have access to two microenvironments: an organelle capable of nutrient uptake situated in close contact with the adsorbing surface while much of the stalk and the entire cell body surface would be bathed in the water flowing past the surface. The cell would not be in close contact with its competitors on the surface and would have access to passing nutrients, including dissolved oxygen, as well as access to metabolic products diffusing away from the periphytic community.

Schmidt (235) suggested that "prosthecae may serve multiple functions, or perhaps none at all." The first alternative is more appealing, since although traits arise (we all assume) by genetic accident, they are preserved by natural selection according to their contributions to survival. Accordingly, although proposals for the functions and adaptive value of the caulobacter appendage cannot yet be based on conclusive evidence, it is possible to recognize functions it performs and to interpret those functions with respect to survival in the environments in which caulobacters occur so commonly. As interpreted here, not only does the stalk itself have adaptive functions, such as flotation, uptake, and a special type of attachment, but also the regulation of its development and the alternation between morphogenesis and reproduction appear to have evolved as additional adaptations, increasing the ability of caulobacters to survive in environments where a juggernaut style of metabolic activity is inappropriate.

## The Caulobacters' Contribution to Nature

When a predaceous mammal kills, feeds, and leaves its prey, the carcass still contains nutrients. Progressively smaller bites may still be taken by progressively smaller scavenging animals, and the final residue may be cleaned up by microorganisms. So in the biosphere, the more obvious steps in consumption involve first large, easily measurable amounts of organic materials that are progressively reduced in soil and aquatic environments to levels measurable only by physical concentration of large volumes. These vanishingly small amounts, if not mineralized, become fossil carbon, sequestered from metabolic utilization. Heterotrophic organisms able to mineralize organic material in very low concentrations are important to carbon cycling in nature not because they turn over vast quantities of material per unit volume of habitat, but because the total volume of the dilute environment—most of the ocean, in particular—is vast. Like the large chunks, the scattered bits of organic matter of the biosphere must be mineralized if that carbon is to serve in primary production by the capture of solar energy.

Caulobacters are ubiquitous in the dilute regions of the biosphere. Their unusual morphology can be interpreted as an aspect of their special suitability for surviving in such environments as vegetative cells ready to absorb and metabolize organic substances, no matter how meager the supply. In this way, they and other oligotrophs serve as the ultimate scanvengers of

the material that escapes the activities of copiotrophic microbial mineralizers. The wide distribution of caulobacters is evidence that the oligotrophic niche is an important aspect of carbon and energy economy in nature.

## LITERATURE CITED


1. **Agabian, N., M. Evinger, and G. Parker.** 1979. Generation of asymmetry during development: segregation of type-specific proteins in *Caulobacter*. J. Cell Biol. **81**:123–136.
2. **Agabian, N., O. M. Rosen, and L. Shapiro.** 1972. Characterization of a protein acyl kinase from *Caulobacter crescentus*. Biochem. Biophys. Res. Commun. **49**:1690–1698.
3. **Agabian, N., and B. Unger.** 1978. *Caulobacter crescentus* cell envelope: effect of growth conditions on murein and outer membrane protein composition. J. Bacteriol. **133**:987–994.
4. **Agabian-Keshishian, N., and L. Shapiro.** 1970. Stalked bacteria: properties of deoxyribonucleic acid bacteriophage φCbK. J. Virol. **5**:795–800.
5. **Agabian-Keshishian, N., and L. Shapiro.** 1971. Bacterial differentiation and phage infection. Virology **44**:46–53.
6. **Alexander, J. L., and J. D. Jollick.** 1977. Transfer and expression of Pseudomonas plasmid RP1 in Caulobacter. J. Gen. Microbiol. **99**: 325–331.
7. **Amemiya, K., C. W. Wu, and L. Shapiro.** 1977. *Caulobacter crescentus* RNA polymerase: purification and characterization of holoenzyme and core polymerase. J. Biol. Chem. **252**:4157–4165.
8. **Atkinson, D. E.** 1971. Adenine nucleotides as stoichiometric coupling agents in metabolism and as regulatory modifiers: the adenylate energy charge, p. 1–21. *In* H. J. Vogel (ed.), Metabolic pathways, vol. 5. Academic Press, Inc., New York.
9. **Austin, B., S. Garges, B. Conrad, E. E. Harding, R. R. Colwell, U. Simidu, and N. Taga.** 1979. Comparative study of the aerobic, heterotrophic bacterial flora of Chesapeake Bay and Tokyo Bay. Appl. Environ. Microbiol. **37**: 704–714.
10. **Babinchak, J. A., and V. F. Gerencser.** 1976. Bacteriophage typing of the "*Caulobacter* group." Int. J. Syst. Bacteriol. **26**:82–84.
11. **Belyaev, S. S.** 1967. Distribution of the caulobacter group of bacteria in the Volga-Don reservoirs. Mikrobiologiya **36**:157–162.
12. **Belyaev, S. S.** 1968. Methods for the enumeration and isolation of *Caulobacter*. Mikrobiologiya **37**:925–929.
13. **Belyaev, S. S.** 1968. Caulobacter in soils and some reservoirs of the USSR. Vestn. Mosk. Univ. **6**:98–105.
14. **Belyaev, S. S.** 1969. Certain aspects of the ecology of *Caulobacter*. Mikrobiologiya **38**:499–504.
15. **Belyaev, S. S.** 1970. Physiological bases of the ecology of Caulobacter, p. 158–166. *In* Microorganisms in the rural economy. Proceedings of the Second Intercollegiate Science Conference, 1968. Moscow University, Moscow.
16. **Bendis, I., and L. Shapiro.** 1970. Properties of *Caulobacter* ribonucleic acid bacteriophage φCb5. J. Virol. **6**:847–854.
17. **Bendis, I. K., and L. Shapiro.** 1973. Deoxyribonucleic acid-dependent ribonucleic acid polymerase of *Caulobacter crescentus*. J. Bacteriol. **115**:848–857.
18. **Blumenthal, L. K., and S. A. Zahler.** 1962. Index for measurement of synchronization of cell populations. Science **135**:724.
19. **Boatman, E. S., and H. C. Douglas.** 1961. Fine structure of the photosynthetic bacterium *Rhodomicrobium vannielii*. J. Biophys. Biochem. Cytol. **11**:469–483.
20. **Bowers, L. E., R. H. Weaver, E. A. Grula, and O. F. Edwards.** 1954. Studies on a strain of *Caulobacter* from water. I. Isolation and identification of *Caulobacter vibrioides* Henrici and Johnson with emended description. J. Bacteriol. **68**:194–200.
21. **Bradley, D. E.** 1967. Ultrastructure of bacteriophages and bacteriocins. Bacteriol. Rev. **31**: 230–314.
22. **Brinton, C. C., Jr.** 1959. Non-flagellar appendages of bacteria. Nature (London) **183**:782–786.
23. **Buchanan, R. E., and N. E. Gibbons.** 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
24. **Bunt, J. S.** 1961. Blue-green algae: growth. Nature (London) **192**:1274–1275.
25. **Bystrický, V.** 1954. Caulobacter—an unusual contaminant of preparations for the electron microscope. Biologia **9**:566–569.
26. **Carter, R. N., and J. M. Schmidt.** 1976. Fatty acid composition of selected prosthecate bacteria. Arch. Microbiol. **110**:91–94.
27. **Cheung, K. K., and A. Newton.** 1977. Patterns of protein synthesis during development in *Caulobacter crescentus*. Dev. Biol. **56**:417–425.
28. **Cheung, K. K., and A. Newton.** 1978. Polyadenylic acid synthesis activity of purified DNA-dependent RNA polymerase from *Caulobacter*. J. Biol. Chem. **253**:2254–2261.
29. **Chow, T. C., and J. M. Schmidt.** 1974. Fatty acid composition of *Caulobacter crescentus*. J. Gen. Microbiol. **83**:369–373.
30. **Clegg, S., and D. C. Old.** 1979. Fimbriae of *Escherichia coli* K-12 strain AW405 and related bacteria. J. Bacteriol. **137**:1008–1012.
31. **Cohen-Bazire, G., R. Kunisawa, and J. S. Poindexter.** 1966. The internal membranes of *Caulobacter crescentus*. J. Gen. Microbiol. **42**: 301–308.
32. **Cohen-Bazire, G., W. R. Sistrom, and R. Y. Stanier.** 1957. Kinetic studies of pigment synthesis by non-sulfur purple bacteria. J. Cell. Comp. Physiol. **49**:25–68.
33. **Conti, S. F., and P. Hirsch.** 1965. Biology of budding bacteria. III. Fine structure of *Rhodomicrobium* and *Hyphomicrobium* spp. J. Bacteriol. **89**:503–512.
34. **Contreras, I., R. A. Bender, J. Mansour, S.**

**Henry, and L. Shapiro.** 1979. *Caulobacter crescentus* mutant defective in membrane phospholipid synthesis. J. Bacteriol. **140**:612–619.

35. **Contreras, I., L. Shapiro, and S. Henry.** 1978. Membrane phospholipid composition of *Caulobacter crescentus*. J. Bacteriol. **135**:1130–1136.
36. **Contreras, I., A. Weissborn, K. Amemiya, J. Mansour, S. Henry, L. Shapiro, and R. Bender.** 1980. The effect of termination of membrane phospholipid synthesis on cell-dependent events in *Caulobacter*. J. Mol. Biol. **138**:401–409.
37. **Corpe, W. A.** 1974. Periphytic marine bacteria and the formation of microbial film on solid surfaces, p. 397–417. *In* R. Colwell and R. Morita (ed.), Effect of the ocean environment on microbial activities. University Park Press, Baltimore.
38. **Corpe, W. A.** 1980. Microbial surface components involved in adsorption of microorganisms onto surfaces, p. 105–144. *In* G. Bitton and K. C. Marshall (ed.), Adsorption of microorganisms to surfaces. John Wiley & Sons, Inc., New York.
39. **Coulton, J. W., and R. G. E. Murray.** 1977. Membrane-associated components of the bacterial flagellar apparatus. Biochim. Biophys. Acta **465**:290–310.
40. **Coulton, J. W., and R. G. E. Murray.** 1978. Cell envelope associations of *Aquaspirillum serpens* flagella. J. Bacteriol. **136**:1037–1049.
41. **Croffy, B. R., and C. S. Stachow.** 1976. Requirement for cellular associations in the development of *Caulobacter crescentus*. Biochem. Biophys. Res. Commun. **71**:307–311.
42. **Datta, N., R. W. Hedges, E. J. Shaw, R. B. Sykes, and M. H. Richmond.** 1971. Properties of an R factor from *Pseudomonas aeruginosa*. J. Bacteriol. **108**:1244–1249.
43. **Dawes, E. A.** 1976. Endogenous metabolism and the survival of starved prokaryotes. Symp. Soc. Gen. Microbiol. **26**:19–53.
44. **deBoer, W. E., and B. J. Spit.** 1964. A new type of bacterial cell wall structure revealed by replica technique. Antonie van Leeuwenhoek J. Microbiol. Serol. **30**:239–248.
45. **deBont, J. A. M., J. T. Staley, and H. S. Pankratz.** 1970. Isolation and description of a non-motile, fusiform, stalked bacterium, a representative of a new genus. Antonie van Leeuwenhoek J. Microbiol. Serol. **36**:397–407.
46. **Degnen, S. T., and N. R. Morris.** 1973. Deoxyribonucleic acid methylation and development in *Caulobacter bacteroides*. J. Bacteriol. **116**: 48–53.
47. **Degnen, S. T., and A. Newton.** 1972. Dependence of cell division on the completion of chromosome replication in *Caulobacter crescentus*. J. Bacteriol. **110**:852–856.
48. **Degnen, S. T., and A. Newton.** 1972. Chromosome replication during development in *Caulobacter crescentus*. J. Mol. Biol. **64**:671–680.
49. **dePamphlis, M. L., and J. Adler.** 1971. Fine structure and isolation of the hook-basal body complex of flagella from *Escherichia coli* and *Bacillus subtilis*. J. Bacteriol. **105**:384–395.
50. **Desjardins, P. R.** 1968. Stalk structure in a *Caulobacter* sp. as revealed by negative staining for electron microscopy. Trans. Am. Microsc. Soc. **87**:392–394.
51. **Dimmott, K., and M. I. Simon.** 1971. Purification and partial characterization of *Bacillus subtilis* flagellar hooks. J. Bacteriol. **108**:282–286.
52. **Donachie, W. D., N. C. Jones, and R. Teather.** 1973. The bacterial cell cycle. Symp. Soc. Gen. Microbiol. **23**:9–44.
53. **Dow, C. S., D. Westmacott, and R. Whittenbury.** 1976. Ultrastructure of budding and prosthecate bacteria, p. 187–221. *In* R. Fuller and D. W. Lovelock (ed.), Microbial ultrastructure. Academic Press, Inc., (London), Ltd., London.
54. **Driggers, L. J., and J. M. Schmidt.** 1970. Induction of defective and temperate bacteriophages in *Caulobacter*. J. Gen. Virol. **6**:421–427.
55. **Duguid, J. P.** 1959. Fimbriae and adhesive properties in Klebsiella strains. J. Gen. Microbiol. **21**:271–286.
56. **Duguid, J. P., and J. W. Wilkinson.** 1961. Environmentally induced changes in bacterial morphology. Symp. Soc. Gen. Microbiol. **11**: 69–99.
57. **Ely, B.** 1979. Transfer of drug resistance factors to the dimorphic bacterium *Caulobacter crescentus*. Genetics **91**:371–380.
58. **Ely, B., A. B. C. Amarasinghe, and R. A. Bender.** 1978. Ammonia assimilation and glutamate formation in *Caulobacter crescentus*. J. Bacteriol. **133**:225–230.
59. **Ely, B., and R. C. Johnson.** 1977. Generalized transduction in *Caulobacter crescentus*. Genetics **87**:391–399.
60. **Evinger, M., and N. Agabian.** 1977. Envelope-associated nucleoid from *Caulobacter crescentus* stalked and swarmer cells. J. Bacteriol. **132**:294–301.
61. **Evinger, M., and N. Agabian.** 1979. *Caulobacter crescentus* nucleoid: analysis of sedimentation behavior and protein composition during the cell cycle. Proc. Natl. Acad. Sci. U.S.A. **76**: 175–178.
62. **Farrell, E. T., and P. R. Smith.** 1978. Nonrandom origin of partial revertants of a pleiotropic phage resistant variant of *Caulobacter crescentus* CB15. J. Gen. Microbiol. **108**:89–95.
63. **Farrell, E. T., and P. R. Smith.** 1978. A study of spontaneous and N-methyl-N′-nitro-N-nitrosoguanidine-induced phage resistant mutants in *Caulobacter crescentus*. J. Gen. Microbiol. **108**:161–164.
64. **Fauré-Fremiet, E.** 1950. Caulobactéries épizoiques associées aux *Centrophorella* (ciliés holotriches). Bull. Soc. Zool. Fr. **75**:134–137.
65. **Freese, E., T. Ichikawa, Y. K. Oh, E. B. Freese, and C. Prasad.** 1974. Deficiencies or excesses of metabolites interfering with differ-

entiation. Proc. Natl. Acad. Sci. U.S.A. **71:** 4188–4193.

66. **Fridovich, I.** 1978. The biology of oxygen radicals. Science **201:**875–880.
67. **Fujiki, K., A. Fukuda, and Y. Okada.** 1976. Amino acid composition of peptidoglycan in *Caulobacter crescentus*. J. Biochem. (Tokyo) **80:**1453–1455.
68. **Fujiki, K., A. Fukuda, and Y. Okada.** 1978. The absence of translational barrier between *Caulobacter crescentus* and *Escherichia coli*. FEBS Lett. **91:**81–84.
69. **Fujiki, K., A. Fukuda, and Y. Okada.** 1978. The terminal bases of ribonucleic acid from *Caulobacter* RNA phage $\phi$Cp2. J. Biochem. (Tokyo) **84:**231–234.
70. **Fukuda, A., H. Iba, and Y. Okada.** 1977. Stalkless mutants of *Caulobacter crescentus*. J. Bacteriol. **131:**280–287.
71. **Fukuda, A., S. Koyasu, and Y. Okada.** 1978. Characterization of two flagella-related proteins from *Caulobacter crescentus*. FEBS Lett. **95:**70–75.
72. **Fukuda, A., K. Miyakawa, H. Iba, and Y. Okada.** 1976. A flagellotropic bacteriophage and flagella formation in *Caulobacter*. Virology **71:**583–592.
73. **Fukuda, A., K. Miyakawa, H. Iida, and Y. Okada.** 1976. Regulation of polar surface structures in *Caulobacter crescentus*: pleiotropic mutations affect the coordinate morphogenesis of flagella, pili and phage receptors. Mol. Gen. Genet. **149:**167–173.
74. **Fukuda, A., K. Miyakawa, and Y. Okada.** 1974. A pleiotropic mutation in *Caulobacter crescentus* that affects the flagellar formation and susceptibility to phage infection. Proc. Jpn. Acad. **50:**839–842.
75. **Fukuda, A., and Y. Okada.** 1977. Effect of macromolecular synthesis on the coordinate morphogenesis of polar surface structures in *Caulobacter crescentus*. J. Bacteriol. **130:** 1199–1205.
76. **Galdiero, F.** 1973. The growth and partition of cell membranes during synchronized division cycle of *Caulobacter crescentus*. Arch. Mikrobiol. **94:**125–132.
77. **Goodwin, S. D., and J. G. Shedlarski.** 1975. Purification of cell wall peptidoglycan of the dimorphic bacterium *Caulobacter crescentus*. Arch. Biochem. Biophys. **170:**23–36.
78. **Gräf, V. W.** 1962. Über Wassermyxobakterien. Arch. Hyg. Bakteriol. **146:**114–125.
79. **Greenawalt, J. W., and T. L. Whiteside.** 1975. Mesosomes: membranous bacterial organelles. Bacteriol. Rev. **39:**405–463.
80. **Gromov, B. V.** 1964. Bacteria of the genus Caulobacter in association with algae. Mikrobiologiya **33:**298–305.
81. **Grula, E. A., and S. E. Hartsell.** 1954. Intracellular structures in *Caulobacter vibrioides*. J. Bacteriol. **68:**498–504.
82. **Grula, E. A., R. H. Weaver, and O. F. Edwards.** 1954. Studies on a strain of *Caulobacter* from water. II. Nutrition, with implications for cytology. J. Bacteriol. **68:**201–206.
83. **Haars, E. G., and J. M. Schmidt.** 1974. Stalk formation and its inhibition in *Caulobacter crescentus*. J. Bacteriol. **120:**1409–1416.
84. **Helmstetter, C., S. Cooper, O. Pierucci, and E. Revelas.** 1968. On the bacterial life sequence. Cold Spring Harbor Symp. Quant. Biol. **33:**809–822.
85. **Helmstetter, C. E.** 1969. Methods for studying the microbial division cycle, p. 327–363. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 1. Academic Press, Inc., London.
86. **Henrici, A. T., and D. E. Johnson.** 1935. Studies on freshwater bacteria. II. Stalked bacteria, a new order of schizomycetes. J. Bacteriol. **30:** 61–93.
87. **Higgins, M. L., H. C. Tsien, and L. Daneo-Moore.** 1976. Organization of mesosomes in fixed and unfixed cells. J. Bacteriol. **127:**1519–1523.
88. **Hirsch, P.** 1974. Budding bacteria. Annu. Rev. Microbiol. **28:**391–444.
89. **Hirsch, P.** 1979. Life under conditions of low nutrient concentrations, p. 357–372. *In* M. Shilo (ed.), Strategies of microbial life in extreme environments. Dahlem Konferenzen Life Sciences Research Report 13. Verlag Chemie, Weinheim, West Germany.
90. **Hirsch, P., and S. F. Conti.** 1964. Biology of budding bacteria. I. Enrichment, isolation and morphology of *Hyphomicrobium* spp. Arch. Mikrobiol. **48:**338–357.
91. **Hirsch, P., and S. F. Conti.** 1965. Enrichment and isolation of stalked and budding bacteria (*Hyphomicrobium, Rhodomicrobium* and *Caulobacter*). Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Suppl. **1:**100–110.
92. **Hirsch, P., and S. H. Pankratz.** 1970. Study of bacterial populations in natural environments by use of submerged electron microscope grids. Z. Allg. Mikrobiol. **10:**589–605.
93. **Houwink, A. L.** 1951. *Caulobacter* versus *Bacillus* spec. div. Nature (London) **168:**654–655.
94. **Houwink, A. L.** 1952. Contamination of electron microscope preparations. Experientia **8:**385.
95. **Houwink, A. L.** 1955. *Caulobacter*. Its morphogenesis, taxonomy and parasitism. Antonie van Leeuwenhoek J. Microbiol. Serol. **21:**49–64.
96. **Houwink, A. L., and W. van Iterson.** 1950. Electron microscopical observations on bacterial cytology. II. A study on flagellation. Biochim. Biophys. Acta **5:**10–44.
97. **Hund, A., and O. Kandler.** 1956. Zur Ernährungsweise und Physiologie von *Caulobacter*. Arch. Mikrobiol. **25:**65–89.
98. **Iba, H., A. Fukuda, and Y. Okada.** 1975. Synchronous cell differentiation in *Caulobacter crescentus*. Jpn. J. Microbiol. **19:**441–446.
99. **Iba, H., A. Fukuda, and Y. Okada.** 1977. Chromosome replication in *Caulobacter crescentus* growing in a nutrient broth. J. Bacteriol. **129:** 1192–1197.
100. **Iba, H., A. Fukuda, and Y. Okada.** 1977. Gamma-ray sensitivity during synchronous cell differentiation in *Caulobacter crescentus*. J.

Bacteriol. **131**:369–371.
101. **Iba, H., A. Fukuda, and Y. Okada.** 1978. Rate of major protein synthesis during the cell cycle of *Caulobacter crescentus*. J. Bacteriol. **135**: 647–655.
102. **Iba, H., and Y. Okada.** 1980. Chromosome segregation in an asymmetrically dividing bacterium, *Caulobacter crescentus*. J. Mol. Biol. **139**:733–739.
103. **Iino, T.** 1969. Genetics and chemistry of bacterial flagella. Bacteriol. Rev. **33**:454–475.
104. **Ikehara, K., H. Kakitani, S. Ishino, and Y. Okada.** 1979. Properties of unprimed poly(A)-poly(U) synthesis by *Caulobacter crescentus* RNA polymerase. J. Biochem. (Tokyo) **85**: 633–640.
105. **Inouye, M., and M. L. Yee.** 1973. Homogeneity of envelope proteins of *Escherichia coli* separated by gel electrophoresis in sodium dodecyl sulfate. J. Bacteriol. **113**:304–312.
106. **Jannasch, H. W., and G. E. Jones.** 1959. Bacterial populations in sea water as determined by different methods of enumeration. Limnol. Oceanogr. **4**:128–139.
107. **Jannasch, H. W., and G. E. Jones.** 1960. *Caulobacter* sp. in sea water. Limnol. Oceanogr. **5**: 432–433.
108. **Jannasch, H. W., and C. O. Wirsen.** 1979. Chemosynthetic primary production at East Pacific sea floor spreading centers. BioScience **29**:592–598.
109. **Johnson, R. C., and B. Ely.** 1977. Isolation of spontaneously derived mutants of *Caulobacter crescentus*. Genetics **86**:25–32.
110. **Johnson, R. C., and B. Ely.** 1979. Analysis of nonmotile mutants of the dimorphic bacterium *Caulobacter crescentus*. J. Bacteriol. **137**:627–634.
111. **Johnson, R. C., M. P. Walsh, B. Ely, and L. Shapiro.** 1979. Flagellar hook and basal complex of *Caulobacter crescentus*. J. Bacteriol. **138**:984–989.
112. **Johnson, R. C., N. B. Wood, and B. Ely.** 1977. Isolation and characterization of bacteriophages for *Caulobacter crescentus*. J. Gen. Virol. **37**:323–335.
113. **Jollick, J. D.** 1972. Differential phage sensitivity of cell types in *Caulobacter*. J. Gen. Virol. **16**: 405–407.
114. **Jollick, J. D., and V. F. Gerencser.** 1973. The mechanism of a host-dependent abortive bacteriophage infection in *Caulobacter*. Can. J. Microbiol. **19**:467–473.
115. **Jollick, J. D., and E. M. Schervish.** 1972. Genetic recombination in *Caulobacter*. J. Gen. Microbiol. **73**:403–407.
116. **Jollick, J. D., and T. Q. Tran.** 1975. Polarity of gene transfer in *Caulobacter*. J. Gen. Microbiol. **91**:183–187.
117. **Jollick, J. D., and B. L. Wright.** 1974. A flagella specific bacteriophage for *Caulobacter*. J. Gen. Virol. **22**:197–205.
118. **Jones, D. E., and J. D. Smith.** 1979. Phospholipids of the differentiating bacterium *Caulobacter crescentus*. Can. J. Biochem. **57**:424–428.
119. **Jones, H. C., and J. M. Schmidt.** 1973. Ultrastructural study of crossbands occurring in the stalks of *Caulobacter crescentus*. J. Bacteriol. **116**:466–470.
120. **Jones, H. E., and P. Hirsch.** 1968. Cell wall composition of *Hyphomicrobium* species. J. Bacteriol. **96**:1037–1041.
121. **Jones, M.** 1905. A peculiar microorganism showing rosette formation. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 2 **14**:459–463.
122. **Jordan, T. L., J. S. Porter, and J. L. Pate.** 1974. Isolation and characterization of prosthecae of *Asticcacaulis biprosthecum*. Arch. Microbiol. **96**:1–16.
123. **Kagawa, H., K. Owaribe, S. Asakura, and N. Takahashi.** 1976. Flagellar hook protein from *Salmonella* SJ25. J. Bacteriol. **125**:68–73.
124. **Kaiser, D., C. Manoil, and M. Dworkin.** 1979. Myxobacteria: cell interactions, genetics, and development. Annu. Rev. Microbiol. **33**:595–639.
125. **Kandler, O., and C. Zehender.** 1956. Papierchromatographische Untersuchung der Aminosäurezusammensetzung vershiedener Bakterien-Hydrolysate. Arch. Mikrobiol. **24**:41–48.
126. **Kandler, O., C. Zehender, and O. Huber.** 1954. Über das Vorkommen von *Caulobacter* spec. in destilliertem Wasser. Arch. Mikrobiol. **21**: 57–59.
127. **Khavina, E. S., and Y. I. Rautenstein.** 1963. Bacteriophage from cultures of bacteria of the genus *Caulobacter*. Dokl. Akad. Nauk. SSSR **153**:197–199.
128. **Koplow, J., and H. Goldfine.** 1974. Alterations in the outer membrane of the cell envelope of heptose-deficient mutants of *Escherichia coli*. J. Bacteriol. **117**:527–543.
129. **Koyasu, S., A. Fukuda, and Y. Okada.** 1980. The penicillin-binding proteins of *Caulobacter crescentus*. J. Biochem. (Tokyo) **87**:363–366.
130. **Krasil'nikov, N. A., and S. S. Belyaev.** 1967. Distribution of Caulobacter in certain soils. Mikrobiologiya **36**:1083–1086.
131. **Krasil'nikov, N. A., and S. S. Belyaev.** 1970. Morphology and development of *Caulobacter*. Mikrobiologiya **39**:352–356.
132. **Krasil'nikov, N. A., and S. S. Belyaev.** 1971. Physiological properties of the bacteria belonging to the Caulobacter genus. Mikrobiologiya **40**:876–879.
133. **Krasil'nikov, N. A., and S. S. Belyaev.** 1971. The growth of Caulobacter in mixed cultures. Mikrobiologiya **40**:1010–1014.
134. **Krasil'nikov, N. A., and S. S. Belyaev.** 1973. Taxonomy and classification of the genus *Caulobacter*. Izv. Akad. Nauk SSSR Ser. Biol. **3**: 313–323.
135. **Kuenen, J. G., J. Boonstra, H. G. J. Schroder, and H. Veldkamp.** 1977. Competition for inorganic substrates among chemoorganotrophic and chemolithotrophic bacteria. Microb. Ecol. **3**:119–130.
136. **Kurn, N., S. Ammer, and L. Shapiro.** 1974. A pleiotropic mutation affecting expression of polar development events in *Caulobacter cres-*

*centus*. Proc. Natl. Acad. Sci. U.S.A. **71**:3157–3161.

137. **Kurn, N., I. Contreras, and L. Shapiro.** 1978. Galactose catabolism in *Caulobacter crescentus*. J. Bacteriol. **135**:517–520.
138. **Kurn, N., and L. Shapiro.** 1975. Regulation of the *Caulobacter* cell cycle. Curr. Top. Cell. Regul. **9**:41–64.
139. **Kurn, N., and L. Shapiro.** 1976. Effect of 3′:5′-cyclic GMP derivatives on the formation of *Caulobacter* surface structures. Proc. Natl. Acad. Sci. U.S.A. **73**:3303–3307.
140. **Kurn, N., L. Shapiro, and N. Agabian.** 1977. Effect of carbon source and the role of cyclic adenosine 3′,5′-monophosphate on the *Caulobacter* cell cycle. J. Bacteriol. **131**:951–959.
141. **Kuznetsov, S. I., G. A. Dubinina, and N. A. Lapteva.** 1979. Biology of oligotrophic bacteria. Annu. Rev. Microbiol. **33**:377–387.
142. **Lagenaur, C., and N. Agabian.** 1976. Physical characterization of *Caulobacter crescentus* flagella. J. Bacteriol. **128**:435–444.
143. **Lagenaur, C., and N. Agabian.** 1977. *Caulobacter crescentus* pili: structure and stage-specific expression. J. Bacteriol. **131**:340–346.
144. **Lagenaur, C., and N. Agabian.** 1977. *Caulobacter* flagellins. J. Bacteriol. **132**:731–733.
145. **Lagenaur, C., and N. Agabian.** 1978. *Caulobacter* flagellar organelle: synthesis, compartmentation, and assembly. J. Bacteriol. **135**: 1062–1069.
146. **Lagenaur, C., M. DeMartini, and N. Agabian.** 1978. Isolation and characterization of *Caulobacter crescentus* flagellar hooks. J. Bacteriol. **136**:795–798.
147. **Lagenaur, C., S. Farmer, and N. Agabian.** 1977. Adsorption properties of stage-specific *Caulobacter* phage φCbK. Virology **77**:401–407.
148. **Lake, J. A., and K. R. Leonard.** 1974. Structure and protein distribution for the capsid of *Caulobacter crescentus* bacteriophage φCbK. J. Mol. Biol. **86**:499–518.
149. **Lake, J. A., and K. R. Leonard.** 1974. Bacteriophage structure: determination of head-tail symmetry mismatch for *Caulobacter crescentus* phage φCbK. Science **183**:744–747.
150. **Lapidus, I. R.** 1978. Time periods of morphological stages in populations of differentiating micro-organisms. J. Theor. Biol. **73**:319–328.
151. **Lapteva, N. A.** 1977. Species composition of heterotrophic bacteria in the water of the Rybinsk Reservoir. Mikrobiologiya **46**:570–577.
152. **Larson, R. J., and J. L. Pate.** 1975. Growth and morphology of *Asticcacaulis biprosthecum* in defined media. Arch. Microbiol. **106**:147–157.
153. **Larson, R. J., and J. L. Pate.** 1976. Glucose transport in isolated prosthecae of *Asticcacaulis biprosthecum*. J. Bacteriol. **126**:282–293.
154. **Leffler, S., M. Hierowski, J. S. Poindexter, and W. Szer.** 1971. Large scale isolation of the Caulobacter bacteriophage φCb5 and its RNA genome. FEBS Lett. **19**:112–114.
155. **Leffler, S., and W. Szer.** 1973. Messenger selection by bacterial ribosomes. Proc. Natl. Acad. Sci. U.S.A. **70**:2364–2368.
156. **Leffler, S., and W. Szer.** 1974. Purification and properties of initiation factor IF-3 from *Caulobacter crescentus*. J. Biol. Chem. **249**:1458–1464.
157. **Leffler, S., and W. Szer.** 1974. Polypeptide chain initiation in *Caulobacter crescentus* without initiation factor IF-1. J. Biol. Chem. **249**:1465–1468.
158. **Leifson, E.** 1960. Atlas of bacterial flagellation. Academic Press, Inc., New York.
159. **Leifson, E.** 1962. The bacterial flora of distilled and stored water. I. General observations, techniques and ecology. Int. Bull. Bacteriol. Nomencl. Taxon. **12**:133–153.
160. **Leifson, E.** 1962. The bacterial flora of distilled and stored water. II. *Caulobacter vibrioides* Henrici & Johnson 1935 in distilled water. Int. Bull. Bacteriol. Nomencl. Taxon. **12**:155–159.
161. **Leifson, E., B. J. Cosenza, R. Murchelano, and R. C. Cleverdon.** 1964. Motile marine bacteria. I. Techniques, ecology, and general characteristics. J. Bacteriol. **87**:652–666.
162. **Leonard, K. R., A. K. Kleinschmidt, N. Agabain-Keshishian, L. Shapiro, and J. V. Maizel, Jr.** 1972. Structural studies on the capsid of *Caulobacter crescentus* bacteriophage φCbK. J. Mol. Biol. **71**:201–216.
163. **Leonard, K. R., A. K. Kleinschmidt, and J. A. Lake.** 1973. *Caulobacter crescentus* bacteriophage φCbK: structure and *in vitro* self-assembly of the tail. J. Mol. Biol. **81**:349–365.
164. **Lindberg, A. A.** 1973. Bacteriophage receptors. Annu. Rev. Microbiol. **27**:205–241.
165. **Loeffler, F.** 1890. Weitere Untersucherung über die Beizung und Färbung der Geisselen bei den Bakterien. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. **7**:625–639.
166. **Lugtenberg, B., R. Peters, H. Bernheimer, and W. Berendsen.** 1976. Influence of cultural conditions and mutations on the composition of outer membrane proteins of *Escherichia coli*. Mol. Gen. Genet. **147**:251–262.
167. **Mandel, M.** 1966. Deoxyribonucleic acid base composition in the genus *Pseudomonas*. J. Gen. Microbiol. **43**:273–292.
168. **Mandel, M., P. Hirsch, and S. F. Conti.** 1972. Deoxyribonucleic acid base compositions of hyphomicrobia. Arch. Mikrobiol. **81**:289–294.
169. **Mandel, M., E. R. Leadbetter, N. Pfennig, and H. G. Trüper.** 1971. Deoxyribonucleic acid base compositions of phototrophic bacteria. Int. J. Syst. Bacteriol. **21**:222–230.
170. **Manoil, C., and D. Kaiser.** 1980. Purine-containing compounds, including cyclic adenosine 3′,5′-monophosphate, induce fruiting of *Myxococcus xanthus* by nutritional imbalance. J. Bacteriol. **141**:374–377.
171. **Mansour, J. D., S. Henry, and L. Shapiro.** 1980. Differential membrane phospholipid synthesis during the cell cycle of *Caulobacter crescentus*. J. Bacteriol. **141**:262–269.
172. **Marino, W., S. Ammer, and L. Shapiro.** 1976. Conditional surface structure mutants of *Caulobacter crescentus*. Temperature-sensitive

flagella formation due to altered flagellin monomer. J. Mol. Biol. **107**:115–130.
173. **Marmur, J., S. Falkow, and M. Mandel.** 1963. New approaches to bacterial taxonomy. Annu. Rev. Microbiol. **17**:329–372.
174. **Marshall, K. C.** 1976. Interfaces in microbial ecology. Harvard University Press, Cambridge, Mass.
175. **Masuda, S.** 1957. Studies on the *Caulobacter* in Japan. J. Jpn. Bot. **32**:321–331.
176. **Matin, A., C. Veldhuis, V. Stegeman, and M. Veenhuis.** 1979. Selective advantage of a *Spirillum* sp. in a carbon-limited environment. Accumulation of poly-β-hydroxybutyric acid and its role in starvation. J. Gen. Microbiol. **112**:349–355.
177. **Matin, A., and H. Veldkamp.** 1978. Physiological basis of the selective advantage of a *Spirillum* sp. in a carbon-limited environment. J. Gen. Microbiol. **105**:187–197.
178. **Middleton, C. A., and J. L. Pate.** 1976. Isolation and partial characterization of some new bacteriophages active against *Asticcacaulis* strains. Int. J. Syst. Bacteriol. **26**:269–277.
179. **Mishustina, I. E., E. Y. Rossova, and M. V. Baturina.** 1976. Electron microscopy in the study of bacterial populations and ultramicroscopic forms in marine waters. Izv. Akad. Nauk SSSR Ser. Biol. **6**:840–848.
180. **Miyakawa, K., A. Fukuda, Y. Okada, K. Furuse, and I. Watanabe.** 1976. Isolation and characterization of RNA phages for *Caulobacter crescentus*. Virology **73**:461–467.
181. **Moaledj, K.** 1978. Qualitative analysis of an oligocarbophilic aquatic microflora in the Plussee. Arch. Hydrobiol. **82**:98–113.
182. **Moore, R. L.** 1977. Ribosomal ribonucleic acid cistron homologies among *Hyphomicrobium* and various other bacteria. Can. J. Microbiol. **23**:478–481.
183. **Moore, R. L., and R. Brubaker.** 1976. Effect of *cis*-platinum(II)diamminodichloride on cell division of *Hyphomicrobium* and *Caulobacter*. J. Bacteriol. **125**:317–323.
184. **Moore, R. L., and P. Hirsch.** 1972. Deoxyribonucleic acid base sequence homologies of some budding and prosthecate bacteria. J. Bacteriol. **110**:256–261.
185. **Moore, R. L., J. Schmidt, J. Poindexter, and J. T. Staley.** 1978. Deoxyribonucleic acid homology among the caulobacters. Int. J. Syst. Bacteriol. **28**:349–353.
186. **Morris, J. G.** 1976. Oxygen and the obligate anaerobe. J. Appl. Bacteriol. **40**:229–244.
187. **Murakami, A., T. Matsuda, N. Watanabe, and S. Nagasawa.** 1976. Degradation of n-paraffin mixtures by marine microorganisms in enriched seawater medium. J. Oceanogr. Soc. Jpn. **32**:242–248.
188. **Nakamura, K., R. M. Pirtle, and M. Inouye.** 1979. Homology of the gene coding for outer membrane lipoprotein within various gram-negative bacteria. J. Bacteriol. **137**:595–604.
189. **Neidhardt, F. C., P. L. Block, and D. F. Smith.** 1974. Culture medium for enterobacteria. J. Bacteriol. **119**:736–747.
190. **Nemec, P., and V. Bystrický.** 1962. Electron microscopy of objects of unusual appearance occurring in the biocenose of diatomaceae. Naturwissenschaften **14**:335.
191. **Nemec, P., and V. Bystrický.** 1962. Peculiar morphology of some microorganisms accompanying diatomaceae. Preliminary report. J. Gen. Appl. Microbiol. **8**:121–129.
192. **Nemec, P., and V. Bystrický.** 1963. Kopulation bei *Caulobacter*. Biol. Zentralbl. **82**:595–600.
193. **Newton, A.** 1972. Role of transcription in the temporal control of development in *Caulobacter crescentus*. Proc. Natl. Acad. Sci. U.S.A. **69**:447–451.
194. **Newton, A., and E. Allebach.** 1975. Gene transfer in *Caulobacter crescentus*: polarized inheritance of genetic markers. Genetics **80**:1–11.
195. **Newton, A., M. A. Osley, and B. Terrana.** 1975. *Caulobacter crescentus*: a model for the temporal and spatial control of development, p. 442–452. *In* D. Schlessinger (ed.), Microbiology—1975. American Society for Microbiology, Washington, D.C.
196. **Nikitin, D. I., and S. I. Kuznetsov.** 1967. Electron microscopic study of the microflora of water. Mikrobiologiya **36**:938–941.
197. **Nisen, P., R. Medford, J. Mansour, M. Purucker, A. Skalka, and L. Shapiro.** 1979. Cell-cycle-associated rearrangement of inverted repeat DNA sequences. Proc. Natl. Acad. Sci. U.S.A. **76**:6240–6244.
198. **Nisen, P., M. Purucker, and L. Shapiro.** 1979. Deoxyribonucleic acid sequence homologies among bacterial insertion sequence elements and genomes of various organisms. J. Bacteriol. **140**:588–596.
199. **Ohta, N., M. Sanders, and A. Newton.** 1975. Poly(adenylic acid) sequences in the RNA of *Caulobacter crescentus*. Proc. Natl. Acad. Sci. U.S.A. **72**:2343–2346.
200. **Ohta, N., M. Sanders, and A. Newton.** 1978. Characterization of unstable poly(A)-RNA in *Caulobacter crescentus*. Biochim. Biophys. Acta **517**:65–75.
201. **Olsen, R. H., and P. Shipley.** 1973. Host range and properties of the *Pseudomonas aeruginosa* R factor R1822. J. Bacteriol. **113**:772–780.
202. **Omeliansky, V. L.** 1914. A new bacillus: *Bacillus flagellatus*. Zh. Mikrobiol. Epidemiol. Immunobiol. **1**:24.
203. **Osborn, M. J.** 1969. Structure and biosynthesis of the bacterial cell wall. Annu. Rev. Biochem. **38**:501–538.
204. **Osborn, M. J., J. E. Gander, E. Parisi, and J. Carson.** 1972. Mechanism of the assembly of the outer membrane of *Salmonella typhimurium*. J. Biol. Chem. **247**:3962–3972.
205. **Osley, M. A., and A. Newton.** 1974. Chromosome segregation and development in *Caulobacter crescentus*. J. Mol. Biol. **90**:359–370.
206. **Osley, M. A., and A. Newton.** 1977. Mutational analysis of developmental control in *Caulobacter crescentus*. Proc. Natl. Acad. Sci. U.S.A.

74:124–128.
207. **Osley, M. A., and A. Newton.** 1978. Regulation of cell cycle events in asymmetrically dividing cells: functions required for DNA initiation and chain elongation in *Caulobacter crescentus*. J. Bacteriol. **135**:10–17.
208. **Osley, M. A., and A. Newton.** 1980. Temporal control of the cell cycle in *Caulobacter crescentus*: roles of DNA chain elongation and completion. J. Mol. Biol. **138**:109–128.
209. **Osley, M. A., M. Sheffery, and A. Newton.** 1977. Regulation of flagellin synthesis in the cell cycle of *Caulobacter*: dependence on DNA replication. Cell **12**:393–400.
210. **Palleroni, N. J., R. Kunisawa, R. Contopoulou, and M. Doudoroff.** 1973. Nucleic acid homologies in the genus *Pseudomonas*. Int. J. Syst. Bacteriol. **23**:333–339.
211. **Pastan, I., and S. Adhya.** 1976. Cyclic adenosine 5'-monophosphate in *Escherichia coli*. Bacteriol. Rev. **40**:527–551.
212. **Pastan, I., and R. Perlman.** 1970. Cyclic adenosine monophosphate in bacteria. Science **169**: 339–344.
213. **Pate, J. L., and E. J. Ordal.** 1965. The fine structure of two unusual stalked bacteria. J. Cell Biol. **27**:133–150.
214. **Pate, J. L., S. J. Petzold, and T. H. Umbreit.** 1979. Two flagellotropic phages and one pilus-specific phage active against *Asticcacaulis biprosthecum*. Virology **94**:24–37.
215. **Pate, J. L., J. S. Porter, and T. L. Jordan.** 1973. *Asticcacaulis biprosthecum* sp. nov. Life cycle, morphology and cultural characteristics. Antonie van Leeuwenhoek J. Microbiol. Serol. **39**:569–583.
216. **Perfil'ev, B. V., and D. R. Gabe.** 1961. Capillary methods of investigating microorganisms. Akad. Nauk SSSR.
217. **Pierucci, O.** 1978. Phospholipid synthesis during the cell division cycle of *Escherichia coli*. J. Bacteriol. **138**:453–460.
218. **Poindexter, J. S.** 1964. Biological properties and classification of the *Caulobacter* group. Bacteriol. Rev. **28**:231–295.
219. **Poindexter, J. S.** 1974. Genus *Caulobacter* Henrici and Johnson 1935, 83, p. 153–155. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
220. **Poindexter, J. S.** 1974. Genus *Asticcacaulis* Poindexter 1964, 288, p. 155–156. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
221. **Poindexter, J. S.** 1978. Selection for nonbuoyant morphological mutants of *Caulobacter crescentus*. J. Bacteriol. **135**:1141–1145.
222. **Poindexter, J. S.** 1979. Morphological adaptation to low nutrient concentrations, p. 341–356. *In* M. Shilo (ed.), Strategies of microbial life in extreme environments. Dahlem Konferenzen Life Sciences Research Report 13. Verlag Chemie, Weinheim, West Germany.
223. **Poindexter, J. S., and G. Cohen-Bazire.** 1964. The fine structure of stalked bacteria belonging to the family Caulobacteraceae. J. Cell Biol. **23**:587–597.
224. **Poindexter, J. S., P. R. Hornack, and P. A. Armstrong.** 1967. Intracellular development of a large DNA bacteriophage lytic for *Caulobacter crescentus*. Arch. Mikrobiol. **59**:237–246.
225. **Poindexter, J. S., and R. F. Lewis.** 1966. Recommendations for revision of the taxonomic treatment of stalked bacteria. Int. J. Syst. Bacteriol. **16**:377–382.
226. **Porter, J. S., and J. L. Pate.** 1975. Prosthecae of *Asticcacaulis biprosthecum*: system for the study of membrane transport. J. Bacteriol. **122**:976–986.
227. **Riley, R. G., and B. J. Kolodziej.** 1976. Pathway of glucose catabolism in *Caulobacter crescentus*. Microbios **16**:219–226.
228. **Romanenko, V. I., M. A. Pubienes, and A. S. Daukshta.** 1978. Growth of bacteria and their activity in surface water film under experimental conditions. Mikrobiologiya **47**:149–157.
229. **Rucinsky, T. E., and E. H. Cota-Robles.** 1974. Mesosome structure in *Chromobacterium violaceum*. J. Bacteriol. **118**:717–724.
230. **Schade, S. Z., J. Adler, and H. Ris.** 1967. How bacteriophage $\chi$ attacks motile bacteria. J. Virol. **1**:599–609.
231. **Schliefer, K. H., and O. Kandler.** 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol. Rev. **36**: 407–477.
232. **Schmidt, J. M.** 1966. Observations on the adsorption of caulobacter bacteriophages containing ribonucleic acid. J. Gen. Microbiol. **45**: 347–353.
233. **Schmidt, J. M.** 1968. Stalk elongation in mutants of *Caulobacter crescentus*. J. Gen. Microbiol. **53**:291–298.
234. **Schmidt, J. M.** 1969. *Caulobacter crescentus* mutants with short stalks. J. Bacteriol. **98**:816–817.
235. **Schmidt, J. M.** 1971. Prosthecate bacteria. Annu. Rev. Microbiol. **25**:93–110.
236. **Schmidt, J. M.** 1973. Effect of lysozyme on crossbands in stalks of *Caulobacter crescentus*. Arch. Mikrobiol. **89**:33–40.
237. **Schmidt, J. M., and G. M. Samuelson.** 1972. Effects of cyclic nucleotides and nucleoside triphosphates on stalk formation in *Caulobacter crescentus*. J. Bacteriol. **112**:593–601.
238. **Schmidt, J. M., and R. Y. Stanier.** 1965. Isolation and characterization of bacteriophages active against stalked bacteria. J. Gen. Microbiol. **39**:95–107.
239. **Schmidt, J. M., and R. Y. Stanier.** 1966. The development of cellular stalks in bacteria. J. Cell Biol. **28**:423–436.
240. **Schmidt, J. M., and J. R. Swafford.** 1975. Ultrastructure of crossbands in prosthecae of *Asticcacaulis* species. J. Bacteriol. **124**:1601–1603.
241. **Schmitt, R., I. Raska, and F. Mayer.** 1974. Plain and complex flagella of *Pseudomonas*

*rhodos*: analysis of fine structure and composition. J. Bacteriol. **117**:844–857.

242. **Schnaitman, C., D. Smith, and M. Forn de Salvas.** 1975. Temperate bacteriophage which causes the production of a new major outer membrane protein by *Escherichia coli*. J. Virol. **15**:1121–1130.
243. **Schnaitman, C. A.** 1970. Protein composition of the cell wall and cytoplasmic membrane of *Escherichia coli*. J. Bacteriol. **104**:890–901.
244. **Schnaitman, C. A.** 1970. Comparison of the envelope protein compositions of several gram-negative bacteria. J. Bacteriol. **104**:1404–1405.
245. **Schnaitman, C. A.** 1974. Outer membrane proteins of *Escherichia coli*: IV. Differences in outer membrane proteins due to strain and cultural differences. J. Bacteriol. **118**:454–464.
246. **Shapiro, L.** 1976. Differentiation in the *Caulobacter* cell cycle. Annu. Rev. Microbiol. **30**: 377–407.
247. **Shapiro, L., and N. Agabian-Keshishian.** 1970. Specific assay for differentiation in the stalked bacterium *Caulobacter crescentus*. Proc. Natl. Acad. Sci. U.S.A. **67**:200–203.
248. **Shapiro, L., N. Agabian-Keshishian, and I. Bendis.** 1971. Bacterial differentiation. Science **173**:884–892.
249. **Shapiro, L., N. Agabian-Keshishian, A. Hirsch, and O. M. Rosen.** 1972. Effect of dibutyryladenosine 3′:5′-cyclic monophosphate on growth and differentiation in *Caulobacter crescentus*. Proc. Natl. Acad. Sci. U.S.A. **69**: 1225–1229.
250. **Shapiro, L., and I. Bendis.** 1975. RNA phages of bacteria other than *E. coli*, p. 397–410. *In* N. Zinder (ed.), RNA phages. Cold Spring Harbor Monograph Series. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
251. **Shapiro, L., and J. V. Maizel, Jr.** 1973. Synthesis and structure of *Caulobacter crescentus* flagella. J. Bacteriol. **113**:478–485.
252. **Shedlarski, J. G., Jr.** 1974. Glucose-6-phosphate dehydrogenase from *Caulobacter crescentus*. Biochim. Biophys. Acta **358**:33–43.
253. **Sheffery, M., and A. Newton.** 1977. Reconstitution and purification of flagellar filaments from *Caulobacter crescentus*. J. Bacteriol. **132**:1027–1030.
254. **Sheffery, M., and A. Newton.** 1979. Purification and characterization of a polyhook protein from *Caulobacter crescentus*. J. Bacteriol. **138**:575–583.
255. **Shehata, T. E., and A. G. Marr.** 1971. Effect of nutrient concentration on the growth of *Escherichia coli*. J. Bacteriol. **107**:210–216.
256. **Shine, J., and L. Dalgarno.** 1975. Determinant of cistron specificity in bacterial ribosomes. Nature (London) **254**:34–38.
257. **Smith, R. W., and H. Koffler.** 1971. Bacterial flagella. Adv. Microb. Physiol. **6**:219–339.
258. **Staley, J. T.** 1968. *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. J. Bacteriol. **95**:1921–1942.
259. **Staley, J. T.** 1971. Incidence of prosthecate bacteria in a polluted stream. Appl. Microbiol. **22**: 496–502.
260. **Staley, J. T.** 1973. Budding and prosthecate bacteria, p. 29–49. *In* A. I. Laskin and H. A. Lechevalier (ed.), Handbook of microbiology, vol. 1. Organismic microbiology. CRC Press, Cleveland.
261. **Staley, J. T., J. A. M. deBont, and K. deJonge.** 1976. *Prosthecobacter fusiformis* nov. gen. et sp., the fusiform caulobacter. Antonie van Leeuwenhoek J. Microbiol. Serol. **42**:333–342.
262. **Staley, J. T., and T. L. Jordan.** 1973. Crossbands of *Caulobacter crescentus* stalks serve as indicators of cell age. Nature (London) **246**: 155–156.
263. **Staley, J. T., and M. Mandel.** 1973. Deoxyribonucleic acid base composition of *Prosthecomicrobium* and *Ancalomicrobium* strains. Int. J. Syst. Bacteriol. **23**:271–273.
264. **Staley, J. T., K. C. Marshall, and V. B. D. Skerman.** 1980. Budding and prosthecate bacteria from freshwater habitats of various trophic states. Microb. Ecol. **5**:245–251.
265. **Stanier, R. Y., N. J. Palleroni, and M. Doudoroff.** 1966. The aerobic pseudomonads: a taxonomic study. J. Gen. Microbiol. **43**:159–271.
266. **Stanley, P. M., E. J. Ordal, and J. T. Staley.** 1979. High numbers of prosthecate bacteria in pulp mill waste aeration lagoons. Appl. Env. Microbiol. **37**:1007–1011.
267. **Starr, M. P., and V. B. D. Skerman.** 1965. Bacterial diversity: the natural history of selected morphologically unusual bacteria. Annu. Rev. Microbiol. **19**:407–454.
268. **Stove, J. L.** 1965. Isolation of *Caulobacter* spp. from water. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Suppl. **1**:95–99.
269. **Stove, J. L., and R. Y. Stanier.** 1962. Cellular differentiation in stalked bacteria. Nature (London) **196**:1189–1192.
270. **Sun, I., L. Shapiro, and O. M. Rosen.** 1975. A specific cyclic guanosine 3′:5′-monophosphate-binding protein in *Caulobacter crescentus*. J. Biol. Chem. **250**:6181–6184.
271. **Sun, I. C., L. Shapiro, and O. M. Rosen.** 1974. Purification and characterization of guanyl cyclase from *Caulobacter crescentus*. Biochem. Biophys. Res. Commun. **61**:193–203.
272. **Swoboda, U., and C. S. Dow.** 1979. The study of homogeneous populations of *Caulobacter* stalked (mother) cells. J. Gen. Microbiol. **112**: 235–239.
273. **Szer, W., J. M. Hermoso, and S. Leffler.** Ribosomal protein S1 and polypeptide chain initiation in bacteria. Proc. Natl. Acad. Sci. U.S.A. **72**:2325–2329.
274. **Szer, W., and S. Leffler.** 1974. Interaction of *Escherichia coli* 30S ribosomal subunits with MS2 phage RNA in the absence of initiation factors. Proc. Natl. Acad. Sci. U.S.A. **71**:3611–3615.
275. **Tamas, G.** 1975. Horizontally occurring quantitative phytoplankton investigations in Lake Balaton, 1974. Ann. Inst. Biol. (Tihany) Hung. Acad. Sci. **42**:219–279.
276. **Terrana, B., and A. Newton.** 1975. Pattern of

unequal cell division and development in *Caulobacter crescentus*. Dev. Biol. **44**:380–385.

277. **Terrana, B., and A. Newton.** 1976. Requirement of a cell division step for stalk formation in *Caulobacter crescentus*. J. Bacteriol. **128**: 456–462.
278. **Thomas, J. O., A. Kolb, and W. Szer.** 1978. Structure of single-stranded nucleic acids in the presence of ribosomal protein. J. Mol. Biol. **123**:163–176.
279. **Umbreit, T. H., and J. L. Pate.** 1978. Characterization of the holdfast region of wild-type cells and holdfast mutants of *Asticcacaulis biprosthecum*. Arch. Microbiol. **118**:157–168.
280. **van Alphen, W., B. Lugtenberg, and W. Berendsen.** 1976. Heptose-deficient mutants of *Escherichia coli* K-12 deficient in up to three major outer membrane proteins. Mol. Gen. Genet. **147**:263–269.
281. **Veldkamp, H., and H. Jannasch.** 1972. Mixed culture studies with the chemostat. J. Appl. Chem. Biotechnol. **22**:105–123.
282. **West, D., C. Lagenaur, and N. Agabian.** 1976. Isolation and characterization of *Caulobacter crescentus* bacteriophage $\phi$Cd1. J. Virol. **17**: 568–575.
283. **Whittenbury, R., and C. S. Dow.** 1977. Morphogenesis and differentiation in *Rhodomicrobium vannielii* and other budding and prosthecate bacteria. Bacteriol. Rev. **41**:754–808.
284. **Wiggs, J. L., J. W. Bush, and M. J. Chamberlin.** 1979. Utilization of promoter and terminator sites on bacteriophage T7 DNA by RNA polymerases from a variety of bacterial orders. Cell **16**:97–110.
285. **Wireman, J. W., and M. Dworkin.** 1975. Morphogenesis and developmental interactions in myxobacteria. Science **189**:516–523.
286. **Wood, N., A. Rake, and L. Shapiro.** 1976. Structure of *Caulobacter* deoxyribonucleic acid. J. Bacteriol. **126**:1305–1315.
287. **Wood, N. B., and L. Shapiro.** 1975. Morphogenesis during the cell cycle of the prokaryote, *Caulobacter crescentus*. Results Probl. Cell Differ. **7**:133–149.
288. **Yasuzumi, G., T. Yamanaka, S. Morita, Y. Yamamoto, and J. Yokoyama.** 1952. Metabolic chromosomes isolated from blood cell nuclei of various animals. Experientia **8**:218–220.
289. **Zavarzina, N. B.** 1961. A lytic agent in cultures of *Chlorella pyrenoidosa* Pringh. Dokl. Akad. Nauk SSSR **137**:435–437.
290. **Zavarzina, N. B.** 1962. The influence of antibiotics on lysis of cultures of *Chlorella pyrenoidosa* Pringh. Mikrobiologiya **31**:1002–1006.
291. **Zavarzina, N. B.** 1964. Lysis of *Chlorella* cultures in the absence of bacteria. Mikrobiologiya **33**:561–564.
292. **ZoBell, C. E.** 1946. Marine microbiology. Chronica Botanica Co., Waltham, Mass.
293. **Zvyagintsev, D. G., A. F. Pertsovskaya, V. I. Duda, and D. I. Nikitin.** 1969. Electron microscopic investigation of the adsorption of microorganisms to soil particles and minerals. Mikrobiologiya **38**:1091–1095.